

ESTIMATION OF GENETIC PARAMETERS, OPTIMAL TEST DESIGNS AND
PREDICTION OF THE GENETIC MERIT OF CLONAL AND SEEDLING
MATERIAL OF EUCALYPTUS GRANDIS

By

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Dedicated to:

My wife, Astrid

My children, Felipe, Daniel, Andres and Lisa

My mother, Cecilia, aunt Isabel and mother-in-law Mariela

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By

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Obtaining accurate and precise genetic parameter estimates is fundamental to determining breeding strategies, and for choosing genotypes for commercial propagation. Results from survival and growth from seven clonal genetic tests of *Eucalyptus grandis* in Colombia supported the contention of sub-dividing them into three different environments for deployment and breeding purposes. Broad sense heritabilities for survival, individual tree volume and mean annual increment (MAI) tended to increase over time for the three environments, but the trends for height were quite different among environments. The genotype by environment interaction (GxE) for growth traits was moderate in the target environment at six years age, and it is recommended to breed and select for clones that are stable across the range of sites within the target environment. The clonal rankings for MAI at the two extreme sites differed markedly both between

these two sites and between them and the five sites in the target environment. Thus, these sites will require separate clonal test locations and deployment populations. The trends of broad sense heritabilities for mean wood density were unexpected, but GxE interaction of wood density was low pointing out that clonal rankings were stable among the five sites within the target environment. The estimation of genetic gains by two methods, predicted clonal values and the classical formula, gave similar results and showed a great potential to increase productivity in the target environment through selection of top clones.

Strong age-age correlations are present between height and mean annual increment (MAI) at three years age with MAI at six years in these clonal genetic tests suggesting this age as appropriate for early selection. This biological selection age coincides with an “economical” age for selection based on discounted selection efficiency in perpetuity, considering a range of interest rates between 5% and 20%. By using a single tree plot design with six locations and six blocks per location between 80% and 90% of the maximum selection efficiency may be obtained. This type of design allows testing of a large number of genotypes across a reasonable number of locations in a cost-effective manner.

The seedling genetic tests showed the growth potential that can be achieved by the infusion of new genetic material from external sources to the breeding population of Smurfit Carton de Colombia (SCC). The higher heritability for the progeny trials and lack of genetic correlation of growth traits between the seedling trials and the clonal trials suggest the presence of nonadditive variance and “C” effects in the clonal tests. For SCC which is committed to clonal forestry the present results suggest clonal testing of all promising individuals for deployment.

CHAPTER 1 INTRODUCTION

The genus *Eucalyptus* contains over 500 species whose natural distribution is mainly confined to Australia and Indonesia. Successful plantation programs are most often developed in a smaller latitudinal range that varies from continent to continent (Eldridge *et al.* 1994). Throughout the world, *Eucalyptus* wood is utilized for industrial and non-industrial purposes. In particular, industrial production for the pulp and paper market has increased markedly in recent years because of the excellent properties that the wood imparts to printing, writing, and tissue papers (Eldridge *et al.* 1994).

A tentative estimate of the world-wide expanse of eucalypt plantations was 10.0 million hectares in 1990 corresponding to about 23% of the total area of forest plantations in the world (Davendra 1995). *Eucalyptus grandis* Hill ex-Maiden is probably the most widely planted species for industrial wood production, with large forestry plantation programs established in Brazil, India, South Africa and Congo. Its success as an exotic species is due to: (1) its fast growth and short rotations, (2) knowledge of clonal propagation techniques by rooted cuttings, (3) wood uniformity and (4) a high worldwide demand for chemical eucalypt pulp (Eldridge *et al.* 1994, Zobel and Talbert 1984, Zobel 1993).

In Colombia research on *Eucalyptus* species and provenances started on a small scale in the 1970s, followed by plantation forestry of the most commercial species *E.*

grandis, *E. globulus* and *E. tereticornis*. Smurfit Carton de Colombia (SCC), the largest pulp and paper company in the country, uses eucalypts as a short-fiber source. The main species is *E. grandis* managed for a rotation of six years from planting to harvest.

A formal tree improvement program for *E. grandis* began in SCC in 1969 and by 1983 the local land race of "Chupillauta" was producing enough seed for operational plantations. The research program at SCC has as its breeding objectives to improve wood quality and timber yield at rotation age, based on volume growth and wood density as the selection criteria.

At the end of 1986, a clonal propagation program based upon rooted cuttings was initiated with mass selection of 1100 superior trees in 2000 ha of company plantations aged 2.5 to 10 years old (Lambeth *et al.* 1989). From these selections and after screening for sprouting ability and rooting characteristics, 460 donor trees (clones) produced enough cuttings to be included in the first clonal trials (series 2-29). From these trials the best clones for volume growth, straightness and other wood quality traits were recommended for further testing and for the establishment of a clonal seed orchard (Lambeth *et al.* 1994).

A series of clonal trials (series 2-36) containing 65 clones was planted across seven locations in 1991 in order to obtain estimates of genetic parameters and to rank the best clones for the operational program. A breeding strategy for the species was developed (Endo and Lambeth 1992) and progeny tests comprising 67 open-pollinated families from the clonal seed orchard (series 2-71) were planted across three locations to determine the best parents for the next generation of breeding. Two years later the

strategy was reviewed and an open-pollinated base population comprising 238 families in four locations was established in 1995 (series 2-73).

The expansion of the forestry program with *E. grandis* has increased in complexity (i.e., new environments and more plantations), and there is a need for accurate and precise genetic information to support sound decisions in the tree improvement program to maximize genetic gains in a cost-effective manner.

At present SCC has one breeding population of open-pollinated families that also serves as a production population to produce seed for seedlings and rooted cuttings. No genetic parameters have been estimated from the progeny tests of this population and preliminary clonal estimates are incomplete and biased by the effect of GxE (Lambeth *et al.* 1994, Endo and Wright 1993).

Obtaining accurate and precise genetic parameter estimates such as heritabilities, genotype-by-environment interaction, age-age correlations, genetic correlations among traits and variance ratios is fundamental to determining breeding strategies, and for choosing genotypes for commercial propagation (White 1987). In this regard there has been an implementation of new analytical tools in forestry, based on mixed linear models (White *et al.* 1986, Jarvis *et al.* 1995, Borralho 1995a, Araujo *et al.* 1996) that are well suited to handling data from different sources, quality and quantity (White and Hodge 1989). In particular, best linear unbiased prediction (BLUP) of breeding values is available from some statistical packages developed by animal breeders, DFREML (Meyer 1988), MTDFREML (Boldman *et al.* 1993), ASREML (Gilmour *et al.* 1997) that allow the simultaneous estimation of genetic and environmental fixed effects for ranking the parents.

The overall goal of this research is to analyze the existing series of *E. grandis* genetic tests (both seedling tests and clonal tests) for three different purposes: (1) estimation of genetic parameters, (2) determination of optimum experimental designs for future genetic clonal tests and (3) comparisons of performance of seedling and clonal material.

The dissertation is divided into four chapters corresponding to these three purposes. Chapter 1 is a general introduction to the dissertation. In chapter 2 the existing clonal trials (Table 2-1) are analyzed to obtain unbiased estimates of heritabilities and genotype-by-environment interactions for growth traits and wood density. Also a comparison of predicted genetic gains by using either mixed model methods or the classical direct method of estimation is discussed.

In Chapter 3 age-age and trait-trait genetic correlations are estimated for growth traits and wood density. This information is used in a simulation study to determine efficient genetic test designs for clonal selection and to estimate the optimum age at which to select clones. Chapter 4 addresses the evaluation of new infusions to the breeding program at SCC and establishes overall comparisons between performance of seedlings and clones planted as rooted cuttings. The genetic correlation is used to predict the correspondence between the total genetic merit of clones planted as cuttings and breeding values of those same clones estimated from their open-pollinated progeny.

CHAPTER 2

AGE TRENDS OF HERITABILITIES AND GENOTYPE-BY-ENVIRONMENT INTERACTION FROM CLONAL TESTS OF EUCALYPTUS GRANDIS

Introduction

General Background

The clonal program of *Eucalyptus grandis* of Smurfit Carton de Colombia started in 1987 with 1100 trees selected in plantations established with seed from: (1) la Marjulia farm, a seed source from Antioquia, Colombia, (2) an unknown provenance from South Africa, (3) Batanicos farm, a seed source from Cauca, Colombia, originally from an unknown provenance of Australia, and (4) the seed production area of Chupillauta, Cauca, originally a provenance trial. Unfortunately, due to the lack of appropriate records of plantations, it was not possible to trace back the selected trees to their original seed source.

From the first series of clonal trials (series 2-29), the best clones for volume growth, straightness and wood quality traits were selected for further testing and for the establishment of a clonal seed orchard (Lambeth *et al.* 1994). Then in 1991, a second series of clonal tests (series 2-36) with the best 65 clones was planted across seven locations (each location containing 29 to 65 clones), in order to estimate genetic parameters and predict genetic merit of these clones for breeding decisions and the operational propagation program.

The sites selected for the trials represented a broad array of ecological conditions where *E. grandis* is operationally planted. Previous research by the company showed that when *E. grandis* is planted below 1000 m asl or above 2200 m, other *Eucalyptus* species perform better than *E. grandis* (Newman 1981, Cannon 1982, Easley and Lambeth 1989).

Previous Work

In the last twenty years, there has been increasing interest on the part of many *Eucalyptus* breeding programs around the world to develop clonal forestry to enhance both plantation productivity and product uniformity (Lambeth *et al.* 1989, Denison and Kietzka 1993, Bertolucci *et al.* 1995, Araujo *et al.* 1997). In this context, the estimation of basic genetic parameters is crucial in determining the best strategies for clonal breeding and to predict genetic gains from deploying the best clones (Burdon 1992, White 1996).

There has been an appreciable amount of research of *E. grandis* in Brazil, however, most information is contained in unpublished theses or Brazilian journals of limited distribution. Therefore in the case of *E. grandis* there are few reports in the literature regarding genetic parameters for growth and wood quality traits of clonal material (Kageyama and Kikuti 1989, Ikemori 1990, Lambeth *et al.* 1994). A summary of broad sense heritability estimates of several traits in *E. grandis* are shown in Appendix 1. In general, broad sense heritabilities showed a moderate genetic control for growth traits ($H^2=0.22$ to 0.41) and wood density ($H^2=0.30$). However, some of these heritability estimates are based on a single genetic test and are therefore upwardly biased by the presence of genotype-by-environment interaction (Comstock and Moll 1963, Hodge and

White 1992). There are no estimates from multiple sites over many ages, and given the importance of *E. grandis* in world plantations, these estimates are important.

Genotype-by-environment interaction (GxE) in forest tree species arises when the relative performance of genetic entries is not consistent under different environments. Breeders deal with its existence by developing separate breeding or propagation populations for each site type when genotypic rankings change or by selecting genotypes that perform well across many sites (McKeand *et al.* 1990).

There are several ways in which the effects of GxE interactions on breeding programs are evaluated. Some common methods are stability analyses (Finlay and Wilkinson 1963, Lin *et al.* 1986, McKeand *et al.* 1990), methods based on analyses of variance (Yamada 1962) and the type B correlation, defining the genetic correlation of the same trait in two different environments (Burdon 1977, Hodge and White 1992, Lu 1999).

In order to define predictable patterns of GxE, some grouping of the genotypes or environments has been proposed based on their relative similarities in performance (Matheson and Cotterill 1990). In other cases, groups of genotypes or environments are formed according to known environmental factors or indices (Hodge and White 1992).

Given the fact that both the tree improvement and the clonal program of *E. grandis* in Smurfit Carton de Colombia are recent, genotype-by-environment interaction of clones has been incompletely tested. Preliminary estimates in Colombia come from a paired-site clonal trial of 27 clones where GxE for growth traits was non-existent between two environments, with good soil quality at three years (Lambeth *et al.* 1994). Other values of type B genetic correlations for clonal material were estimated at one year of age (Endo and Wright 1993), but the trials were too young to provide useful estimates. Further the results

of the literature are incomplete and surprisingly few are from well-designed clonal tests in multiple locations.

Goals

The objective of this chapter was to use multivariate mixed model methods based on restricted maximum likelihood (REML), to obtain unbiased estimates of genetic parameters for growth traits and wood density from clonal trials assessed from 1 through 6 years, which is the rotation age in Colombia. These estimates included: (1) trends of broad sense heritabilities (H^2) and of genotype-by-environment interaction (GxE) for each traits, and (2) predicted genetic gains at six years of age.

The results of this study provide reliable estimates of age trends in these important traits and give useful insights for planning breeding and clonal deployment programs in Colombia and other countries, where *E. grandis* is planted. Correlations among the various ages and their implications for selection at different ages are the subject of the next chapter.

Materials and Methods

Field Establishment and Test Design

The sites selected for the trials represented not only the target environment for forestry plantations of *E. grandis* in Colombia, but also marginal sites with extreme environmental conditions where *E. grandis* is operationally planted on a limited scale (Table 2-1). Therefore, the assessment of the productivity at these sites provides valuable information for management decisions.

Table 2-1. Location and climatic conditions of clonal tests, series 2-36, of *Eucalyptus grandis* in Smurfit Carton de Colombia

Location	Test	Latitude (N)	Longitude (O)	Altitude (m)	Mean Annual Rainfall (mm)	Mean Annual Temperature (°c)
Arcadia	1	2° 29'	76° 40'	1750	2155	18.3
Cedral	2	4° 42'	75° 40'	1850	2820	17.0
Guachicono	3	3° 34'	76° 28'	950	1052	24.0
Indostan	4	4° 15'	75° 47'	1750	2414	18.3
Maravillas	5	4° 20'	75° 38'	2400	2340	13.0
La Tulia	6	4° 22'	76° 20'	1970	2000	17.0
Suiza	7	3° 49'	76° 28'	1560	1156	18.9

Mass propagation of the best 65 clones selected from the first clonal tests (study 2-29) was done at the "Restrepo" nursery, Department of Valle, and the management of the sprouts, cuttings and rooted plants was similar to operational clones in the nursery. Site preparation and cultural practices were basically the same at all sites and followed the standard practices applied by the operational plantation program (Table 2-2).

The experimental design at each location was a randomized complete block design with variable numbers of blocks and 6-tree row plots with a spacing of 3x3 m (Table 2-2). The variable number of clones and blocks at each site is related to the quantity of ramets available from each clone and to the relative importance of clonal testing in new plantation areas (site 2), in areas close to the mill (site 3), or highly productive areas (site 7). The study is unbalanced with 27 clones common to all sites and almost all clones are in three sites.

Data Collection

Survival, height and diameter were measured at ages one through six and wood density at three and six years. Total height was measured with a telescopic pole at ages

one and two and with a hypsometer from years three to six. Diameter at breast height (DBH) was assessed from ages two to six using diameter tapes. Basic wood density was sampled by randomly selecting three trees from each family in each block and extracting, at breast height, 5mm wood increment cores. Basic wood density was estimated by the water volume displacement method (ASTM 1969).

Table 2-2. Site preparation and layout of clonal tests of *Eucalyptus grandis* at Smurfit Carton de Colombia. Fertilization was at time of planting and rates shown are amounts of fertilizer applied per tree.

Location	Test	Blocks (No)	Clones (No)	Site Preparation	Fertilization (g/tree)
Arcadia	1	6	29	Burning	70 NPK (15-38-10) 15 Borax (48%)
Cedral	2	8	64	Cleaning, plowing herbicide	70 NPK (15-38-10) 10 Borax (48%)
Guachicona	3	2	65	Burning, plowing and disking	50 MAP – 50 SNH ₄ 15 Borax (68%)
Indostan	4	6	29	Burning, herbicide	70 NPK (15-38-10) 15 Borax (48%)
Maravillas	5	6	29	Herbicide	50 MAP – 50 DAP 15 Borax (68%)
La Tulia	6	5	30	Herbicide	70 NPK (15-38-10) 15 Borax (48%)
Suiza	7	8	64	Burning, herbicide	70 NPK (15-38-10) 10 Borax (48%)

Notes: MAP = mono-ammonium phosphate, SNH₄ = ammonium sulfate
NPK = compound fertilizer with Nitrogen, Phosphate and Potassium.

Individual tree volume and mean annual increment (MAI) were estimated at ages two to six. Individual tree volume, overbark, determinations were made according to the following volume equation developed for clones by the Planning Department of Smurfit Carton de Colombia (Uribe 1995):

$$\text{Volume (m}^3\text{)} = 0.024 + 0.335 D^2H$$

Mean annual increment (MAI) was estimated by multiplying each tree volume by the corresponding plot survival and expressing it on a mean annual basis ($\text{m}^3 \text{ha}^{-1} \text{yr}^{-1}$).

Data Editing and Standardization

Prior to analyses, editing of the data was performed to remove measurements of recording errors. The identification of potential outliers and influential measurements was conducted by building a linear regression for height and diameter at each site using PROC INSIGHT (SAS® 1993). This procedure allowed the plotting of all observations and provided useful information on diagnostic measurements for outliers (Myers 1990) as well as their effects on the moments of the distribution i.e., mean, error variance, skewness, and kurtosis (Sorensen and White 1988). In all, fewer than 2% of the observations per block were deleted from the data based not only on their statistical information, but also on the physical and biological observations supplied at the time of measurements.

In order to remove scale effects and help to create homogeneous variance structures across tests, the square root of the block phenotypic variance was used to standardize all continuous traits before the analyses (Falconer 1993, White 1996). That is, for each growth and wood density trait, each tree's measurement was divided by the phenotypic standard deviation of its corresponding block producing a transformed variable with phenotypic variance of one.

Preliminary Analyses

Before analyses, the data were examined for any factors affecting the performance of individual trees. The most relevant factor affecting growth was the stem breakage of trees due to the strong winds during the dry seasons, January-February and June-July, at

“Arcadia”, “Cedral”, and “Maravillas” (Appendix 2-2a). Broken-topped trees resumed growth soon after the damage occurred and only a few died. The effect of strong winds has been also reported for *E. grandis*, *E. urophylla* and their hybrids (Wang and Yang 1995) in China and for *Pinus tecunumanii* (Schw.) progeny trials in Colombia (Dvorak *et al.* 1993).

Analyses of variance using broken-tops as a covariable were performed on height, and on the ratio DBH/height, between broken-topped trees and recovered trees from previous damage. These analyses were performed to provide information leading to the use of either similar or different volume equations for the broken-topped trees. The analyses were conducted using PROC GLM (SAS® Institute 1990) and based on raw data. Broken topped trees were recorded as zero (0) and normal trees were recorded as one (1). The linear model used for the analyses is presented in Appendix 2.

It was also of interest to know if stem breakage was under genetic control, therefore, variance components were obtained by running single site analyses and a pooled analysis for the three sites with high frequencies of this trait. Variance components were estimated using PROC MIXED (Littell *et al.* 1996), according to the models showed in Appendix 3. Estimates of individual broad sense heritability of stem breakage for single and pooled sites were made based on the formulae presented in Falconer (1993).

Preliminary analyses including or not including broken-topped trees gave similar estimates for all growth and wood density traits, therefore, all results hereafter reported included all trees.

Analyses of Survival, Growth Traits and Wood Density

Prior studies on the performance of *E. grandis* on areas similar to the tests sites (Cannon 1982, Easley and Lambeth 1989), and preliminary analyses using pairwise comparisons of all test combinations (Appendix 4), showed that the two sites "Guachicona" and "Maravillas" were distinctly different from the other five sites.

The "Guachicona" and "Maravillas" sites are extreme environments and different from the other five sites, which represent the target environment for Smurfit Carton de Colombia (Table 2-1). Further, even though "Guachicona" and "Maravillas" are potentially important for the company they represent less than 5% of the total area planted with *E. grandis*. This situation led to the use of a multivariate analysis that considers each analyzed variable as three traits: (1) "Guachicona", (2) "Maravillas" and (3) a target environment, defined by the remaining five sites that is representative of 95% of the area with *E. grandis* forests.

Estimates of variance components for survival (%), total height (m), individual tree volume (m^3), MAI ($\text{m}^3\text{ha}^{-1}\text{year}^{-1}$) and wood density (gcm^{-3}), were obtained from the multivariate computer program MTDFREML (Boldman *et al.* 1993). Multiple Trait Derivative-Free Restricted Maximum Likelihood, MTDFREML, is a set of programs based on a derivative-free algorithm that can be used for single or multiple-trait models, with or without repeated observations (Boldman *et al.* 1993).

MTDFREML uses restricted maximum likelihood (REML) to estimate variance components based on residuals calculated after fitting the fixed effects of the model by generalized least squares (Searle *et al.* 1992). REML estimation is the preferred choice in

animal breeding (Henderson 1984) and it has also proven to have better properties for unbalanced data, in forest genetic tests, than other estimators (Huber *et al.* 1994).

Multivariate methods allow simultaneous estimation of variances and covariances of different traits in a closed system, yielding empirically unbiased estimates of type B genetic correlations for unbalanced data with heterogeneous variances (Lu 1999). Moreover, for multivariate models MTDFREML provides estimates of variance component and covariance component estimates that are constrained within the parameter space, facilitating their interpretation and practical applications (Lu 1999).

For the derivative-free method, the solution for variance or covariance components occurs when the global maximum of the log likelihood function is found. The variance component estimates from MTDFREML were verified by univariate analyses of each trait using PROC MIXED ® (Littell *et al.* 1996).

In this study, survival was analyzed based on 0/1 scores, without transformation, given that enough evidence has been produced to support the fact that REML analysis on 0/1 data provides satisfactory results (Banks *et al.* 1985, Westfall 1987, Huber 1993, Dieters *et al.* 1996, Lopes 1998). Other growth traits and wood density were standardized before the analyses as was previously described.

The statistical analysis of each variable followed the multivariate mixed model, in matrix form:

$$\begin{aligned}
\begin{bmatrix} y_1 & - & - \\ - & y_2 & - \\ - & - & y_3 \end{bmatrix} &= \begin{bmatrix} X_1 & 0 & 0 \\ 0 & X_2 & 0 \\ 0 & 0 & X_3 \end{bmatrix} \begin{bmatrix} b_1 & - & - \\ - & b_2 & - \\ - & - & b_3 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 & 0 \\ 0 & Z_2 & 0 \\ 0 & 0 & Z_3 \end{bmatrix} \begin{bmatrix} c_1 & - & - \\ - & c_2 & - \\ - & - & c_3 \end{bmatrix} \\
&+ \begin{bmatrix} N_1 & - & - \\ 0 & - & - \\ 0 & - & - \end{bmatrix} \begin{bmatrix} d_1 & - & - \\ - & - & - \\ - & - & - \end{bmatrix} + \begin{bmatrix} Q_1 & - & - \\ 0 & - & - \\ 0 & - & - \end{bmatrix} \begin{bmatrix} p_1 & - & - \\ - & - & - \\ - & - & - \end{bmatrix} \\
&+ \begin{bmatrix} - & 0 & 0 \\ - & W_2 & 0 \\ - & 0 & W_3 \end{bmatrix} \begin{bmatrix} - & - & - \\ - & t_2 & - \\ - & - & t_3 \end{bmatrix} + \begin{bmatrix} e_1 & - & - \\ - & e_2 & - \\ - & - & e_3 \end{bmatrix} \quad 2-2
\end{aligned}$$

where

y_1 = vector of observations corresponding to trait 1, the target environment with five sites; y_1 has measurements for the target environment and is missing for the other two environments.

y_2 = vector of observations corresponding to trait 2, "Guachicon"; y_2 has measurements for "Guachicon" and is missing for the other two environments.

y_3 = vector of observations corresponding to trait 3, "Maravillas"; y_3 has measurements for "Maravillas" and is missing for the other two environments.

b = matrix of fixed effects for site and block effects for trait 1 ($i=1$), and block fixed effects for traits 2 and 3 ($i=2,3$).

c = matrix of random clonal effects corresponding to traits 1, 2, and 3 and c is $MVN \sim (0, C)$. C is a square matrix and has dimensions equivalent to $j \times j$ with

$$j = \sum_{i=1}^3 N_i \text{ and } N_i \text{ is the total number of clones } (c).$$

d = vector of the uncorrelated random effects clone by site interactions, and d is $N \sim (0, D)$. D is a square matrix ($\sigma_{cs}^2 I_k$) and has dimensions equivalent to $k \times k$ with

k equal to the total number of combinations of clone by site (cs) for trait 1, i.e. only present in the model for trait 1.

p = vector of the uncorrelated random effects block within site by clone interactions and p is $N \sim (0, K)$. K is a square matrix ($\sigma^2_{bsc} I_l$) and has dimensions equivalent to lx with l equal to the total number of combinations of block within site by clone (bsc) for trait 1, i.e. only present in the model for trait 1.

t = matrix of the uncorrelated random effects block by clone interactions, and t is $MVN \sim (0, T)$. T is a square matrix and has dimensions equivalent to $m \times m$ with m equal to the total number of blocks by clone combinations (bc) for traits 2 and 3 i.e. only present in the model for traits 2 and 3.

e = matrix of random residual effects for traits $i = 1, 2$, and 3 and e is

$MVN \sim (0, R)$. R is a square matrix and has dimensions equivalent to $r \times r$ with r equal to the total number of observation (e) for traits 1, 2 and 3.

The variance and covariance components are:

$$\text{Var} \begin{bmatrix} c_1 & - & - \\ - & c_2 & - \\ - & - & c_3 \end{bmatrix} = \begin{bmatrix} \sigma^2_{c1} & \sigma_{c12} & \sigma_{c13} \\ \sigma_{c21} & \sigma^2_{c2} & \sigma_{c23} \\ \sigma_{c31} & \sigma_{c32} & \sigma^2_{c3} \end{bmatrix} \otimes I_c = C$$

$$\text{Var} [d_1] = [\sigma^2_{cs1} I_{N1cs}] = D$$

$$\text{Var} [p_1] = [\sigma^2_{bsc1} I_{N1bsc}] = K$$

$$\text{Var} \begin{bmatrix} t_2 & - \\ - & t_3 \end{bmatrix} = \begin{bmatrix} \sigma^2_{bc22} I_{N2bc} & 0 \\ 0 & \sigma^2_{bc33} I_{N3bc} \end{bmatrix} = T$$

$$\text{Var} \begin{bmatrix} e_1 & - & - \\ - & e_2 & - \\ - & - & e_3 \end{bmatrix} = \begin{bmatrix} \sigma^2_{e1} I_{N1e1} & 0 & 0 \\ 0 & \sigma^2_{e2} I_{N2e2} & 0 \\ 0 & 0 & \sigma^2_{e3} I_{N3e3} \end{bmatrix} = \mathbf{R}$$

$$\text{Var} \begin{bmatrix} y_1 & - & - \\ - & y_2 & - \\ - & - & y_3 \end{bmatrix} = \mathbf{ZCZ}' + \mathbf{NDN}' + \mathbf{QKQ}' + \mathbf{WTW}' + \mathbf{R} = \mathbf{V}$$

where

X, Z, N, Q and W are incidence matrices relating records to the fixed, clonal and uncorrelated random effects. X, Z, N, Q and W have dimensions equivalent to

$(n \times p)$ with $n = 3 \sum_{i=1}^3 Ni$ and n = the total number of observations corresponding to

traits 1, 2 and 3 and p is the number of levels for the model effects.

σ^2_c , $\sigma^2_{b(c)}$, σ^2_{cs} , σ^2_{bc} , σ^2_e are the clonal variance and the variances of block within site by clone, clone by site, block by clone and residual effects respectively.

σ_{c12} is the covariance of additive effects between traits 1 and 2.

In addition to variance components, MTDFREML provides estimates of broad-sense individual heritabilities for trait 1 (pooled over 5 sites), and for traits 2 and 3 (single-site), of the variables analyzed according to the following equations:

Trait 1, (Pooled sites):

$$H^2 = \frac{\sigma^2_c}{\sigma^2_c + \sigma^2_{b(s)c} + \sigma^2_{cs} + \sigma^2_e} \quad 2-3$$

Traits 2 and 3, (Single site):

$$H^2 = \frac{\sigma^2_c}{\sigma^2_c + \sigma^2_{bc} + \sigma^2_e} \quad 2-4$$

The heritabilities estimates of growth characteristics for traits 2, “Guachiconá”, and 3, “Maravillas” are upwardly biased by the presence of genotype-by-environment interaction (Comstock and Moll 1963). Estimates of heritabilities for survival were obtained on the underlying scale, the liability scale, to reduce the influence of the incidence of the trait and facilitate comparison of estimates from tests with different mean incidences (Dempster and Lerner 1950, Gianola 1982, Lopes 1998). The following equation was used for this purpose:

$$H^2 = H^2_{o1} \frac{p(1-p)}{z^2} \quad 2-5$$

where

H^2_{o1} is the heritability on the observed binomial scale (Appendix 5), p is the incidence of survival in the trait and z is the height of the ordinate at the threshold corresponding to the incidence in the trial (Dempster and Lerner 1950, Gianola 1982, Lopes 1998).

Genotype-by-Environment Interaction

The type B genetic correlation (r_B) for the five sites composing the target environment was calculated with the estimates of variance components obtained from MTDFREML following Yamada (1962):

$$r_{gB} = \frac{\sigma^2_{\epsilon}}{\sigma^2_{\epsilon} + \sigma^2_{cs}} \quad 2-6$$

where

r_B is the type B genetic correlation

σ^2_{ϵ} is the clonal variance in trait 1 from model 2-2 (the 5 sites of the target

environment).

σ^2_{cs} is the variance of the interaction effect of clone by site in trait 1.

In order to understand the factors that influence the growth of *E. grandis*, linear regression analyses of the growth variables on the climatic factors, elevation (m), mean annual temperature (°C), and annual mean precipitation (mm/year) were performed (Appendix 6). Furthermore, the GxE interaction term corresponding to each genotype in each site, obtained from the univariate analysis of MAI in the target environment, was also regressed on the climatic factors to identify those factors that were causing most of the interaction in growth traits.

The discrimination of genotypes that cause most of the interaction was attempted using a modified stability index (Sierra 1999), which expressed the GxE interaction term of each clone as a weighted proportion of the total GxE interaction (Appendix 6). Clones with stability indices above one are very reactive and clones with values close to one or less than one are stable and non-reactive respectively. A similar procedure was followed to identify the most interactive sites by determining their contribution to the total GxE interaction term.

Estimation of Genetic Gains

Genetic gain for individual tree volume and mean annual increment was estimated for the target environment by two methods at six years. The first method used the predicted genetic merit of the clones obtained from the MTDFREML analyses. These “clonal values” were predicted using Best Linear Unbiased Prediction (BLUP), which

considers “clonal values”, as random effects to be predicted rather than fixed effects to be estimated (Henderson 1984, White and Hodge 1988).

The standardized clonal values were back transformed to clonal gains by first multiplying by the square root of the mean phenotypic variance ($\sqrt{\sigma_p^2}$) across all blocks and then dividing by the phenotypic mean ($\bar{x}_p$) of the target environment. Gains were estimated by selecting the best out of the 67 tested and successively increasing by one the number of selections until all trees were selected. This results in 67 different gains predictions at decreasing levels of selection intensity.

The second method to predict genetic gains from clonal selection was made according to Falconer (1996), using the respective heritability of clonal means:

$$\text{Gain (\%)} = H^2_{\bar{c}} * i * \sigma_{p\bar{c}} \quad 2-7$$

The genetic gain was previously adjusted by a factor B to express the standardized values on a percentage basis:

$$B = (\sigma_p / \bar{x}_p) * 100 \quad 2-8$$

where

B is a back-standardization factor

$H^2_{\bar{c}}$ is broad sense heritability based on clone means

i is the intensity of selection

$\sigma_{p\bar{c}}$ is the square root of the phenotypic variance of clonal means

σ_p is the square root of the mean phenotypic variance across all blocks

$\bar{x}_p$ is the phenotypic mean of the target environment

and broad sense heritability of clonal means is calculated based on the clonal phenotypic standard deviation and expressed as:

$$H^2_{\bar{c}} = \frac{\sigma^2_c}{\sigma^2_c + \frac{\sigma^2_{cs}}{s} + \frac{\sigma^2_{b(s)c}}{bs} + \frac{\sigma^2_e}{bsn}} \quad 2-9$$

where

σ^2_c , σ^2_{cs} , σ^2_{bsc} , and σ^2_e are the clonal variance, clone by site interaction variance, block within site by clone interaction variance, and residual variance respectively and,

s = number of sites, r = number of blocks per site and n = harmonic mean of the number of ramets per plot.

Results and Discussion

Stem Breakage

The mean percent stem breakage by wind for *E. grandis* was low in four tests (0% to 3%) and moderate (8.5% to 22%) in the three tests, “Arcadia”, “Cedral”, and “Maravillas”, at six years age (Appendix 2-2a). Single site analyses at three years reflect differences between trees affected that particular year and non-damaged trees, while analyses in later years indicate differences between non-damaged trees at three years and damaged trees recovered in later years.

For the latter tests, the single-site analyses of variance on height indicated non-significant differences ($p < 0.05$) among clones at all ages and highly significant differences ($p < 0.01$) between non-damaged and recovered trees at each specific age (Appendix 2-3). The clone by broken-top interaction was also highly significant

indicating that clones recover differently over time.

The single-site analyses of variance on the ratio DBH/height showed no significant ($p < 0.05$) clonal differences at any ages, except at the “Maravillas” site at 5 years age (Appendix 2). There were also non-significant differences among non-damaged trees and recovered trees at all sites and ages, except at “Cedral” at 6 years age ($p < 0.0489$). The interaction clone by broken-top was highly significant ($p < 0.01$) in “Cedral” and mainly non-significant at “Arcadia” and “Maravillas” at all measurement times.

It is possible that in Cedral the interaction of clone by broken-top is confounded with the damage to the terminal buds caused by the presence of leaf diseases (i.e., *Cylindrocadium spp*), or swelling of the trunk caused by *Chryphonetria cubensis*. These diseases have been shown to decrease the potential growth of specific clones at Cedral.

The previous results suggest that recovered broken-top trees exhibit a similar ratio of DBH/height to unaffected trees and thus the use of one equation for estimation of individual tree volume seems appropriate. Further, the low genetic control present for the trait precludes its inclusion as a selection trait for propagation purposes (Appendix 3).

Average Trends in Survival and Growth

Average survival (%) for the target environment represented by the five sites in the target environment (1, 2, 4, 6, and 7) over the six years, was high and much better than survival at either of the extreme sites of “Guachicono” or “Maravillas” (Figure 2-1). The survival results of *E. grandis* from the five sites of the target environment are in agreement with all previous clonal studies located in representative areas of operational

plantings, which had shown survival greater than 90% at half-rotation (Lambeth *et al.* 1991).

At the “Maravillas” site the survival is a reflection of the poor adaptability of the species to lower temperatures at high elevations, where cold-tolerant *Eucalyptus* species perform better (Newman 1981, Cannon 1982, Easley and Lambeth 1989).

Mortality at “Guachicono” was very high in the first year (16%) and it increased steadily until the fourth year when mortality increased sharply again (5 to 7% per year). Mortality at this site is associated with hot-dry climate and the long-term agricultural practices, irrigation and fertilization, developed in the production of annual crops in this alluvial soil. Most of the deaths at this site are probably related to drought conditions, high pH (around 6.5) and the clay texture of the soil that is associated with low permeability and poor drainage creating unfavorable conditions for root growth.

Good site preparation and a permanent control of competing vegetation during the first 18 months (Ladrach 1983, Endo and Wright 1993) are common practices followed at the company to reduce the risk of mortality and to realize the potential growth of the species.

All growth traits (total height, individual tree volume, and mean annual increment (MAI) showed a consistent pattern, with clones performing better in the target environment, followed by “Guachicono” and “Maravillas”, respectively (Figure 2-1). Height and individual tree volume increased through time over the six years, however, MAI seems to have reached a plateau in the target environment at five years.

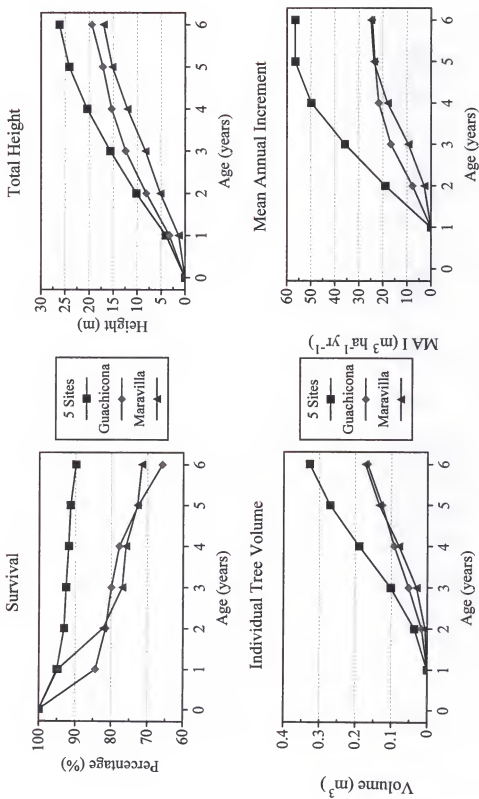


Figure 2-1. Trends of average survival, height, individual tree volume and mean annual increment (MAI) of *Eucalyptus grandis* across time.

The growth rate of clones of *E. grandis* in the target environment through 6 years, for both individual tree volume (0.33 m^3) and MAI ($58 \text{ m}^3\text{ha}^{-1}\text{y}^{-1}$), is in line with other estimates of productivity of the species in the tropics (Ikemori 1990, Zobel 1993, Darrow 1995). However, the estimates for growth at “Guachicono” and “Maravillas” are very marginal with less than half of the growth of the target environment. It would be possible to increase the productivity of *E. grandis* in these sites by hybridizing the species with some other *Eucalyptus* species, such as *E. camaldulensis* and *E. globulus*, that have shown better adaptability and performance in “Guachicono” and “Maravillas”, respectively (Cannon 1982, Ladrach 1987).

Trends of Broad Sense Heritabilities

Survival

Broad sense heritability for survival is reported in the underlying continuous scale after transformation (Figure 2-2), however, estimates based on 0/1 data and the percentage of incidence for the trait at the different environments are presented in Appendix 5.

The results showed that heritability of survival at the “Guachicono” site tended to increase with age and that it was under strong genetic control after the second year ($H^2=0.40$ to 0.50). The heritability for survival at “Maravillas” showed a steady increase during the first three years, when the incidence of survival dropped sharply (23 %), and from this point forward it stabilized exhibiting a moderate genetic control ($H^2=0.26$).

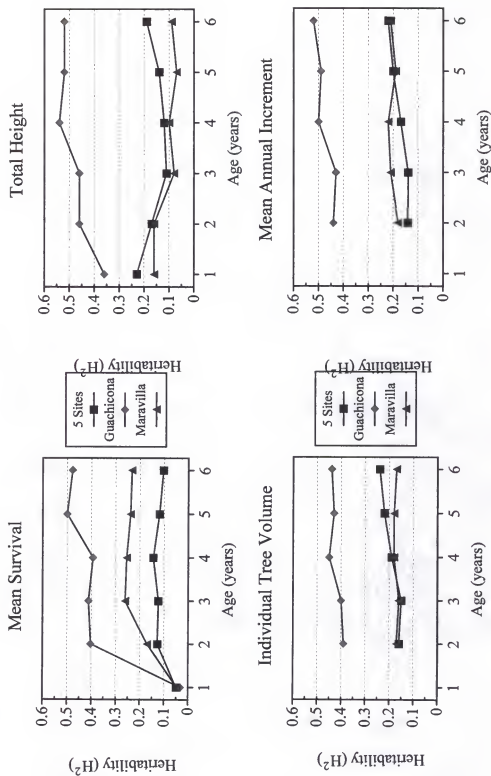


Figure 2-2. Trends of broad sense heritabilities (H^2) for survival, height, individual tree volume and mean annual increment (MAI) of *Eucalyptus grandis* across time.

On the other hand, heritability of survival in the target environment was, in general, low increasing during the first two years and thereafter remaining almost constant between 0.10 and 0.15.

There are almost no published estimates of heritabilities of survival for *E. grandis*. At a very young age, 1.25 years, narrow sense heritability for survival in Florida, in the binomial scale ($h^2=0.09$), was within the range of broad sense heritabilities for six clonal trials $H^2=0.02$ to 0.10 (Rockwood *et al.* 1989).

On the contrary, estimates of heritability of survival in the underlying scale, of open pollinated families of *E. globulus* spp. *globulus* in areas where frost damage was the main cause of mortality (54 – 12%), showed high heritabilities varying from $h^2_{ns}=0.32$ to 0.57 at 4 to 5 years of age (Chambers *et al.* 1996).

The results in “Maravillas”, the cold site, seem to agree at age one with those obtained in Florida, considering that the heritabilities in the binomial scale are biased downward when compared to the underlying scale. However, they oppose the results of *E. globulus* in which the estimates of heritability are high in the cold sites, and exceeded those estimates of heritability ($h^2=0.23 - 21$) in sites with low survival (47 – 63%) and affected by drought (Chambers *et al.* 1996).

Growth traits

As a general result broad sense heritabilities for growth traits were higher at the “Guachicono” site than for either “Maravillas” or the target environment (Figure 2-2). Differences in heritabilities across traits are associated with changes in the magnitude of the variance components (Appendix 7). Lower environmental variation was observed for

all growth traits at "Guachicono" a flat site, versus the other test sites located in gentle slopes in the mountains, probably due to a better layout of the trial in the field or a more positive response to intensive management practices. Furthermore, estimates of heritabilities at "Guachicono" and "Maravillas" are biased upward since the single-site estimates include the clonal variation (σ^2_c) plus the clone by site interaction term (σ^2_{cs}) in the numerator of the equation (2-4) for single-site H^2 (Comstock and Moll 1963).

Broad sense heritability for total height was markedly different for "Guachicono" compared to the other sites. At "Guachicono" the heritability increased during the first four years and then stabilized at a very high level $H^2=0.5$. At "Maravillas" and the target environment the heritability decreased until the third year, and then it leveled off at "Maravillas", but increased until rotation age at the target environment.

Heritabilities for height showed no clear tendency with age, however, some possible factors that alter variance components and heritability estimates of height during the first three years might be related to:

- (1) Trees with broken tops affecting the relative frequencies of genotypes and therefore variances at both "Maravillas" and the target environment (i.e. through the influence of "Arcadia" and "Cedral" sites).
- (2) At "Guachicono", the hot and dry site, competition effects among trees might be different than those in the mountains due to a more uniform spacing, as well as the intensive silviculture (i.e. weed control), which minimized microenvironmental effects, therefore reducing environmental variance.

At "Guachicono" the heritability trend for volume and MAI was similar and

generally increased over time with the estimates for MAI ($H^2=0.44$ to 0.52), being higher than those of volume ($H^2=0.39$ to 0.44). A more stable response was obtained at "Maravillas" where estimates for MAI varied from $H^2=0.18$ to 0.21 and those of volume were almost constant around 0.17 . Therefore, these estimates showed a strong genetic control for both traits in "Guachicona" and only indicated a moderate genetic control at "Maravillas".

Broad-sense heritability estimates for individual tree volume and MAI, were moderate and showed similar trends in the target environment. The estimates slightly decrease or remain constant until year three and thereafter they gradually increased until rotation age. These estimates of broad sense heritabilities for growth traits are lower than those obtained in previous clonal trials (Lambeth *et al.* 1994) because, in the latter, the estimates at each age were averages of single-site analyses and are upwardly biased by the clone by site interaction term.

It seems that when mortality is high as in "Guachicona" and "Maravillas", clonal differences in MAI are exacerbated and the heritability estimates are slightly higher than those estimates of volume at each particular site. On the contrary, the heritability estimates for volume and MAI in the target environment were of similar magnitude due to the lack of a mortality effect.

There was no clear association among trends in heritabilities of both volume and mean annual increment with mortality in the different environments. However, the effects of mortality in the estimation of genetic parameters may be compared to the effects of thinnings on these parameters in progeny trials. By simulation and field data, using diameters (DBH), Matheson and Raymond (1984) demonstrated that random removal of

trees within a plot did not seem to affect the estimates of variance components. On the contrary, a selective elimination of the smaller trees increased genetic variance and decreased phenotypic variance, therefore increasing heritabilities.

Mortality is a function of tree size, vigor, and random environmental effects (Magnussen 1989). Therefore, it might be possible that at "Guachicono" the smaller trees within plots were prone to die more frequently than the bigger trees with high vigor, in this manner reducing the phenotypic variance in the test.

Genotype-by-Environment Interactions

Preliminary analyses of total height (m), individual tree volume (m^3), and MAI ($\text{m}^3\text{ha}^{-1}\text{year}^{-1}$) using data from all possible pairs of sites supported the previous decision of considering the target environment, (5 sites), "Guachicono", and "Maravillas" as three distinct traits (Appendix 4).

Moreover, the genetic correlations derived from the multivariate analyses among "traits" using MTDFREML, are in fact measurements of the GxE interaction among these three site groupings (Table 2-3). These interaction terms indicated a type B genetic correlation of zero between the target environment and either of the other two sites in MAI, and a negative correlation ($r_B = -0.49$) between "Guachicono" and "Maravillas" for MAI at six years. Low to moderate type B genetic correlations among traits were found for height and individual tree volume, respectively.

Genotype-by-environment interaction among the five sites in the target environment over the years was moderate as indicated by the type B genetic correlation of approximately 0.6 for both individual tree volume and MAI (Figure 2-3). The type B

genetic correlation increased until the third year and thereafter it decreased until rotation age. The type B genetic correlation for total height was low in the first year, and then it fluctuated between 0.50 and 0.64 the remaining years.

Table 2-3. Broad-sense heritabilities and genetic correlations among groups of environments treated as different "traits" (1= target environment, 2=Guachicono and 3= Maravillas), for growth variables and wood density of growth analyzed at six years of age.

Trait	Height ¹ Trait			Volume Trait			MAI Trait			Wood Density Trait		
	1	2	3	1	2	3	1	2	3	1	2	3
1	0.19			0.24			0.22			0.35		
2	0.21	0.52		0.54	0.44		0.02	0.52		0.63	0.29	
3	0.20	-0.56	0.09	0.34	0.65	0.17	0.01	-0.49	0.21	0.88	0.81	0.41

Note ¹: Broad-sense heritabilities on diagonals and genetic correlations (r_B) among "traits" below diagonals.

The linear regression analysis of growth traits on climatic data, elevation, mean annual precipitation and mean annual temperature, showed that both elevation and temperature or precipitation and temperature explained only about 5% of the total variability in any growth variable. Besides, the regression analysis of the GxE clonal term on climatic data revealed no association between GxE and the independent variables. These results suggest that growth traits and the GxE interaction effect are influenced by either other climatic factors or soil characteristics. Therefore, more information regarding the environmental conditions where *E. grandis* is planted should be gathered in order to explain the causes for the GxE interaction and to increase gains from clonal deployment.

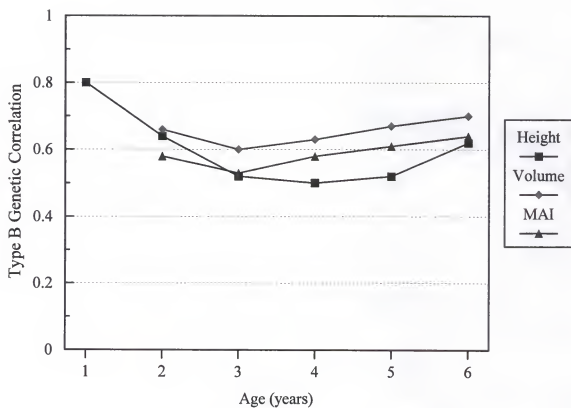


Figure 2-3. Trends of type B genetic correlations for growth traits of *Eucalyptus grandis* at five locations representing the target environment across time.

The analyses to identify the most interactive sites showed that two sites account for 53 % of the total GxE interaction; however no meaningful association between productivity at these sites and the climatic information available was evident. The analyses to identify the most interactive genotypes indicated that 20 out of the 67 clones were very unstable, with stability indices above one, but most clones were contributing to the GxE interaction.

The low predictability of the climatic factors associated with the GxE interaction, and its moderate magnitude for mean annual increment ($r_b=0.64$) at six years, implies the use of a unique clonal propagation population for the “target environment”. The genotype-by-environment interaction for mean annual increment (MAI), even though more conservative than that of volume ($r_b=0.70$), has the advantage of taking into account the mortality effects. Therefore it may better represent the performance of the clones in operational plantations.

Wood Density

Mean values of wood density were very similar for “Maravillas” and the target environment, but lower than those values at “Guachicono” (Figure 2-4). With the exception of the latter, the results compare favorably with other published estimates of wood density at the same age (van Wyk 1990, Wang and Yang 1995, Denison and Kietzka 1993). The high values of wood density at “Guachicono” may be related to the factors limiting growth at the site (Zobel *et al.* 1987), moisture availability, its distribution, and the physical soil conditions.

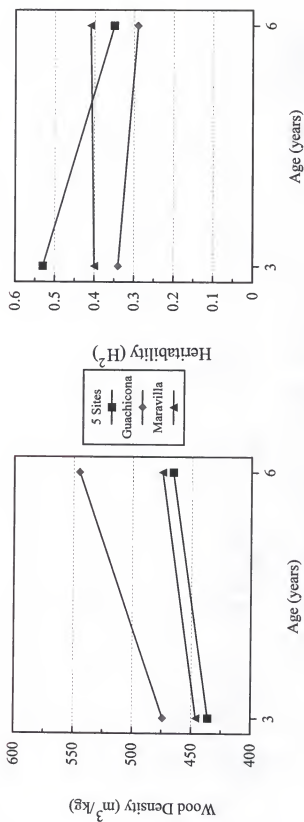


Figure 2-4. Trends of average basic wood density and broad sense heritabilities of seven clonal trials of *Eucalyptus grandis* across time.

Trends in the heritabilities of wood density showed a different pattern for each environment (Figure 2-4). Broad-sense heritability for wood density was stable in “Maravillas”, but it decreased in both “Guachicono” and the target environment over time. Broad-sense heritability in the target environment was high at three years and consistent with other reported estimates of wood density in *Eucalyptus* (Malan 1991). However, the estimate markedly dropped at six years as the clonal variance decreased and the within plot variance increased.

Wood density heritabilities for “Guachicono” and “Maravillas” were of moderate genetic control (Figure 2-4). The large type B genetic correlations of wood density among the three groups of sites, the target environment, “Guachicono” and “Maravillas”, pointed out that this trait could be considered as one trait across all sites, and that some clonal rankings will change between the target environment and “Guachicono” (r_B in Table 2-3).

The genotype-by-environment interaction of wood density within the target environment was unimportant ($r_B=0.90$) at three and six years of age indicating that clonal rankings remain almost the same across all sites in this environment. These results coincide with previous studies (Bhat *et al.* 1990) which revealed very little differences in the wood properties of *E. grandis* between geographical regions. Nevertheless, Malan and Verry (1996) reported changes in wood density ranks with small changes in the environment, for 9 out of 25 *E. grandis* clones.

Genetic Gains

The estimation of clonal genetic gains for mean annual increment and individual tree volume at six years showed, for both methods of estimation, high genetic gains for

intensities of selection above $i=1.0$, corresponding to proportions smaller than 25 clones out of 67, $p=0.37$, (Figure 2-5). Differences between genetic gains, derived from marginal clonal values or from the classical formula, are related to the advantage of BLUP which adjusts all predictions to reflect unbalance in the data while in the standard formula the assumption is made that all clones have the same heritability and equal representation in all tests. Also, gains from the standard formula assume a normal distribution of data, but for mixed model analyses this distribution is not assumed and selections at the end of the tail in the normal distribution can have a different mean than the same number of selections using BLUP. Even with these differences, the two approaches resulted in markedly similar predicted gains at all selection intensities.

Conclusions

Differences in survival, growth rates and type B genetic correlations from the multivariate analyses support the contention of sub-dividing the seven sites into three different groups, a target environment (five sites), “Guachicono” and “Maravillas”. These groups of sites are representative of the planting area of the company. The target environment representing the main planting region, and two extreme sites (“Guachicono”, a hot-dry low-elevation site, and “Maravillas” a cold high-elevation site).

The low type B genetic correlation ($r_b=0.46$) among the extreme sites for survival suggested that mortality may be controlled by different genes in these two different edaphoclimatic conditions. Further, the continued decline in survival over time indicates the poor adaptability of the species to the particular environmental conditions at both sites.

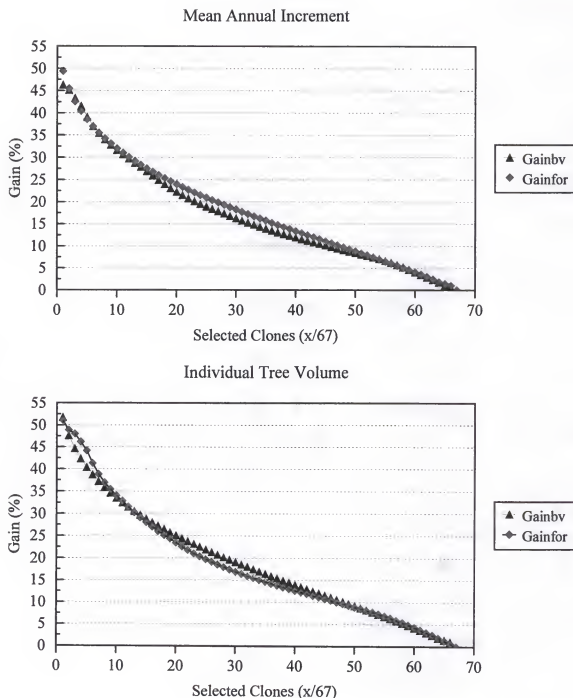


Figure 2-5. Clonal genetic gains in mean annual increment (MAI) and individual tree volume, in the target environment, estimated by using predicted breeding values and the standard formula. Each gain estimate is computed by selecting the best clone out of a total of 67 until selecting all of them. Gainbv stands for gain computed from predicted clonal values and Gainfor refers to gain computed from the standard formula (Falconer and Mackay 1996).

Previous studies have shown better growth of other *Eucalyptus* species, (i.e. *E. camaldulensis*, *E. tereticornis* in the hot-dry conditions of Guachicon and *E. globulus* in the colder Maravillas). Perhaps hybridization of these species with *E. grandis* can capitalize on the good traits of the parental species producing a more competitive product for the mill.

Broad sense heritabilities for survival, individual tree volume and mean annual increment (MAI) tended to increase over time for the three environments, but the trends for height were quite different among environments. Mean annual increment (MAI) seems the most appropriate variable to be used as an indicator of growth and genetic parameters since it incorporates both mortality and growth rate providing a better indicator for operational clonal plantings.

The genotype-by-environment interaction (GxE) for growth traits was moderate ($r_B=0.6$ to 0.7) in the target environment at six years age. Within the target environment, GxE could not be related to any of the climatic factors analyzed making it difficult to choose clones for specific conditions. Rather it will be important to select for clones that are stable and well adapted across the range of edaphoclimatic zones within the target environment. Furthermore, the clonal rankings for MAI at the two extreme sites differed markedly both between those two sites and between them and the target environment. Thus, these sites will require separate clonal test locations and deployment populations.

The trends of broad sense heritabilities for mean wood density were unexpected, heritabilities tended to stay constant or decrease over time for the three environments. GxE interaction of wood density was low ($r_B=0.9$) pointing out that clonal rankings were very stable among the five sites within the target environment.

The estimation of genetic gains by the two methods, predicted clonal values and the classical formula of estimation, gave similar results and showed a great potential to increase productivity in the target environment. However, the method of gains based on clonal values estimated with BLUPs should provide more precise estimates due to the proper accounting for data structure.

CHAPTER 3

AGE-AGE AND TRAIT-TRAIT CORRELATION FOR EUCALYPTUS GRANDIS AND THEIR IMPLICATIONS FOR OPTIMAL SELECTION AGE AND DESIGN OF CLONAL TESTS

Introduction

Increasing interest in developing clonal forestry in *Eucalyptus spp* to enhance both plantation productivity and product uniformity (Araujo *et al.* 1997, Bertolucci *et al.* 1995, Denison and Kietzka 1993, Lambeth *et al.* 1989) requires estimation of basic genetic parameters in order to determine the best strategies for clonal testing and breeding and to predict genetic gains from deploying the best clones (Burdon 1982, White 1996). Some important genetic parameters were estimated in Chapter Two such as heritabilities and genotype-by-environment interaction for growth traits and wood density; these are useful for defining which traits, for estimating gains and for determining breeding, deployment and testing zones. In addition, other genetic parameters are needed for designing genetic tests and determining optimal selection age. In particular age-age and trait-trait correlations have received considerable attention due to their potential to maximize genetic gains and to design breeding strategies.

Tree selection in genetic tests is usually conducted before rotation age with the purpose of minimizing the generation interval and consequently increasing genetic gains per unit of time. Half rotation age is a common age for final assessment of families and individuals (Franklin 1979, Zobel and Talbert 1984) and several studies have been done

to examine selection at a much earlier age (Lambeth 1980, Cotterill and Dean 1988), even in the nursery or in a controlled environment facility (Williams 1988, Surles *et al.* 1993).

Early selection has been the subject of research for most long-lived tree species of economical importance and the literature contains a large number of reports for species such as *Pinus taeda* (Lambeth *et al.* 1983, Foster 1986, McKeand 1988, Balocchi 1994), *Pinus elliottii* (La Farge 1975, White and Hodge 1992), *P. radiata* (Cotterill and Dean 1988, Matheson *et al.* 1994), *Pseudotsuga menziesii* (Riiter and Perry 1987, Bastien and Roman-Amat 1990) and spruce species (Nienstaedt 1984, Mullin and Park 1994). For these species different models have been proposed to predict changes in variance estimates over time (Franklin 1979) or changes in correlations between traits at different ages (Lambeth 1980, Kang 1985, Magnussen 1991).

Most previous research cited above was for longer rotation conifers, however, short-rotation species such as *Eucalyptus*, have also been generating some information on age-age correlations in the last decade from the most commercially planted species such as *E. globulus* (Borrallho *et al.* 1992b), *E. grandis* (van Wyk 1990, Lambeth *et al.* 1994), and *E. nitens* (Greaves *et al.* 1997), *E. urophylla* (Wei and Borrallho 1998), or eucalypt hybrids (Bouvet 1991, Bertolucci *et al.* 1995, Vigneron 1995). However, most estimates are additive genetic correlations from open-pollinated or full-sib progeny. Reports on clonal genetic correlations for *Eucalyptus* are scarce (Ikemori 1990, Bouvet 1991, Lambeth *et al.* 1994) and they only cover the first few years of a rotation.

Optimal selection ages are commonly based either on gains per unit of time (Lambeth *et al.* 1983, Falconer 1993) or by a method that incorporates the time value of money such as in present value analyses in economics (McKeand 1988, Balocchi 1990,

White and Hodge 1992). These latter methods calculate discounted selection efficiencies that penalize (or discount) the value of selection at later ages to various degrees based on an interest or discount rate. The incorporation of the risk associated with juvenile ages has been also explored as a criterion for decision making of the optimum selection age (Newman and Williams 1991).

For *Eucalyptus* species managed on short rotations of six to eight years, optimal selection age based on discounted selection efficiencies (DSE) could be based on an infinite number of generations. This concept, called discounted selection efficiency in perpetuity, would account for the repeated benefits of early selection in all subsequent rotations, assuming that gain is obtained at the end of each generation cycle. To our knowledge, this method has not been used.

The tree improvement and the clonal program of *E. grandis* at Smurfit Carton de Colombia are recent and the information gathered on age-age and trait-trait correlations of the clones is scant and limited to the first years of growth (Moraes 1990, Lambeth *et al.* 1994). Therefore, more precise and reliable correlations between traits and between early ages and the target trait at rotation age are required.

This chapter utilizes data from seven clonal tests of *E. grandis* containing 65 clones to (1) Estimate age-age correlations for height, mean annual increment (MAI) and wood density, between age one through age five, with the target trait MAI at rotation age (six years), (2) Estimate genetic correlations between traits at three and six years age, (3) Determine optimal test designs for clonal selection, and (4) Examine the effect of discounted selection efficiency in perpetuity on selection age. The results of this study

should provide useful insights for planning the breeding, testing and clonal deployment programs of *E. grandis*.

Materials and Methods

Field Test Design and Traits Measured

The experimental design was a randomized complete block design with variable numbers of blocks and 6-tree row plots with a spacing of 3x3 m. Between 29 to 65 clones were planted at each test with 27 common clones to all sites and almost all clones in three sites.

Survival and total height were measured at ages one through six and wood density at three and six years. Diameter at breast height (DBH) was assessed from ages two to six. Basic wood density was sampled by randomly selecting three trees from each family in each block and extracting a 5mm wood increment cores at DBH.

Basic wood density was estimated by the water volume displacement method (ASTM 1969). Individual tree volume, overbark, and mean annual increment (MAI) were estimated at ages two to six as previously described in Chapter 2.

Prior to analyses, editing of the data was performed to remove measurements of recording errors. In all, fewer than 2% of the observations per block were deleted from the data based not only on their statistical information, but also on the physical and biological observations supplied at the time of measurements.

In order to remove scale effects and help to create homogeneous variance structures across tests, the square root of the block phenotypic variance was used to standardize all continuous traits before the analyses (White 1996, Falconer 1993, Gianola

1986). That is, for each growth trait and wood density, each tree's measurement was divided by the phenotypic standard deviation of its corresponding block producing a transformed variable with phenotypic variance of one.

The seven sites of the trials were classified into three distinct groups representing a target environment (five sites in the primary *E. grandis* planting area) and two marginal sites (Guachicona and Maravillas) with extreme ecological conditions where the species has been planted on a minor scale. A brief summary of the environmental conditions and field layout is presented in Table 3-1.

Table 3-1. Climatic conditions and field layout of clonal tests, series 2-36, of *Eucalyptus grandis* planted in three different environments at Smurfit Carton de Colombia.

Location	Tests (#)	Altitude (m)	Mean Annual Rainfall (mm)	Mean Annual Temp. (°c)	Blocks (No)	Clones (No)
Guachicona	1	950	1052	24.0	2	65
Maravillas	1	2400	2340	13.0	6	29
Target Environment	5	1560 – 1970	1156 - 2820	17.0 – 18.9	6 - 8	29 - 65

Estimation of Genetic Correlations

Estimates of variance components and covariance for survival (0/1 data), total height (m), individual tree volume (m^3), MAI ($\text{m}^3\text{ha}^{-1}\text{year}^{-1}$) and wood density (gcm^{-3}) were based on bivariate mixed model equations and obtained from the multivariate computer program MTDFREML (Boldman *et al.* 1993). MTDFREML uses restricted maximum likelihood (REML) to estimate variance components (Searle *et al.* 1992).

Multivariate methods allow simultaneous estimation of variances and covariance of different traits in a closed system, yielding empirically unbiased estimates of type B genetic correlations for unbalanced data with heterogeneous variances (Lu 1999). Moreover, multivariate methods are more efficient than univariate methods and MTDFREML maintains all variance component estimates within the parameter space, facilitating their interpretation and practical applications (Lu 1999).

Linear models for all bivariate analyses, age-age or trait-trait correlations, of five sites in the target environment followed the mixed model, in matrix form:

$$\begin{aligned} \begin{bmatrix} y_1 & y_2 \end{bmatrix} = & \begin{bmatrix} X & Z & N & Q \end{bmatrix} \begin{bmatrix} b_1 & b_2 \\ c_1 & c_2 \\ d_1 & d_2 \\ p_1 & p_2 \end{bmatrix} \\ & + \begin{bmatrix} e_1 & e_2 \end{bmatrix} \end{aligned} \quad 3-1$$

where

y_1 = vector of observations corresponding to trait 1 at an earlier age or any trait,

y_2 = vector of observations corresponding to trait 2 at the target age or any trait,

b_i = vectors of fixed effects for site and block effects for trait i ($i=1,2$),

c_i = vectors of random clonal effects corresponding to traits 1, and 2, and

$c_i \sim \text{MVN}(\mathbf{0}, C)$,

d_i = vector of the uncorrelated random effects corresponding to clone by site interactions, and d_i is $\text{MVN}(\mathbf{0}, D)$,

p_i = vector of the uncorrelated random effects corresponding to block within site by clone interactions and p_i is $\text{MVN}(\mathbf{0}, K)$

e_i = vectors of random residual effects for traits 1 and 2, and e_i is $\text{MVN}(\mathbf{0}, R)$ and

X , Z , N , and Q are incidence matrices relating records to the fixed effects, and

clone, clone by site, and clone by block within site, random effects respectively.

The variance and covariance components are:

$$\text{Var} \begin{bmatrix} c_1 & c_2 \end{bmatrix} = \begin{bmatrix} \sigma^2_{c1} & \sigma_{c12} \\ \sigma_{c12} & \sigma^2_{c2} \end{bmatrix} \otimes I_C = \begin{bmatrix} C_{11} & C_{12} \\ C_{21} & C_{22} \end{bmatrix} = C$$

$$\text{Var} \begin{bmatrix} d_1 & d_2 \end{bmatrix} = \begin{bmatrix} \sigma^2_{cs1} & \sigma_{cs12} \\ \sigma_{cs12} & \sigma^2_{cs2} \end{bmatrix} \otimes I_{CS} = \begin{bmatrix} D_{11} & D_{12} \\ D_{21} & D_{22} \end{bmatrix} = D$$

$$\text{Var} \begin{bmatrix} p_1 & p_2 \end{bmatrix} = \begin{bmatrix} \sigma^2_{bsc1} & \sigma_{bsc12} \\ \sigma_{bsc12} & \sigma^2_{bsc2} \end{bmatrix} \otimes I_{bsc} = \begin{bmatrix} K_{11} & K_{12} \\ K_{21} & K_{22} \end{bmatrix} = K$$

$$\text{Var} \begin{bmatrix} e_1 & e_2 \end{bmatrix} = \begin{bmatrix} \sigma^2_{e1} & \sigma_{e12} \\ \sigma_{e12} & \sigma^2_{e2} \end{bmatrix} \otimes I_N = R$$

$$\text{Var} \begin{bmatrix} y_1 & y_2 \end{bmatrix} = \begin{bmatrix} ZC_{11}Z' & ZC_{12}Z' \\ ZC_{21}Z' & ZC_{22}Z' \end{bmatrix} + \begin{bmatrix} ND_{11}N' & ND_{12}N' \\ ND_{21}N' & ND_{22}N' \end{bmatrix} + \begin{bmatrix} QK_{11}Q' & QK_{12}Q' \\ QK_{21}Q' & QK_{22}Q' \end{bmatrix} + R$$

where

$\sigma^2_{c1}, \sigma^2_{cs1}, \sigma^2_{bsc1}, \sigma^2_{e1}$ are the variances of clonal effects, clone by site, block within site by clone, and residual effects corresponding to trait 1, and

$\sigma_{c12}, \sigma_{cs12}, \sigma_{bsc12}, \sigma_{e12}$ are the covariances of clonal effects, clone by site, block within site by clone, and residual effects between traits, respectively, and

I is an identity matrix with dimensions equal to the observed random variable.

Additional to variance components, MTDFREML provides estimates of genetic, environmental and phenotypic correlations for pairs of traits or ages according to the following equation:

$$r_{xy} = \frac{\sigma_{xy}}{\sqrt{\sigma^2_x \sigma^2_y}}$$

where

r_{xy} is the genetic, environmental or phenotypic correlation between trait x and trait y or for the same trait at different ages

σ_{xy} is the clonal genetic covariance, environmental covariance or phenotypic covariance between two traits or ages and,

σ_x^2 and σ_y^2 are the clonal genetic, environmental or phenotypic variances of trait x and trait y

Broad-sense individual heritabilities for each trait in the bivariate analysis obtained from combined analyses over five sites were calculated by MTDFREML with the estimates of variance components as follows:

$$H^2 = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_{cs}^2 + \sigma_{hsc}^2 + \sigma_e^2} \quad 3-3$$

The statistical analysis for genetic correlations between traits and age-age genetic correlations at Guachicon and Maravillas followed the bivariate mixed model, in matrix form identical to Equation 3-1 except that for these single-site analyses all terms related to site effects and clone by site interactions were deleted (Equation 3-4):

$$[y_1 \ y_2] = [X] [b_1 \ b_2] + [Z] [c_1 \ c_2] + [\emptyset] [p_1 \ p_2] + [e_1 \ e_2] \quad 3-4$$

Additional to variance components, MTDFREML provides estimates of broad-sense individual heritabilities for the single-site analyses according to the following equations:

$$H^2 = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_{hc}^2 + \sigma_e^2} \quad 3-5$$

Optimal Test Designs

A simulation study was conducted to determine optimal genetic test designs for clones in order to improve tree growth (MAI) as a breeding objective. The approach used for the optimal design objective, clonal selection, was a randomized complete block design (RCBD) with a plot configuration of single tree plots (STP) and a variable number of test sites (1 to 10) and blocks (1 to 12) per site.

Single tree plots (STP) have been shown to be more efficient than row plots (Loo-Dinkins and Tauer 1987) and they can be used effectively for both backward and forward selection, allowing not only an efficient ranking of parents but also an effective estimation of genetic parameters (White 1996).

The estimates of broad-sense heritability and type B genetic correlations obtained from the five clonal tests in the target environment were used as a basic set of genetic parameter estimates from which "standard parameter estimates" were developed for the simulation. The approach of standard parameter estimates derives values that may be generally applicable and useful for selection purposes (Cotterill and Dean 1990, White and Hodge 1992). The process begins with observed parameter estimates but then models and smooths the observed estimates so they are internally consistent and seem biologically reasonable.

Estimates of individual tree heritability for mean annual increment (MAI) from ages two through six years were developed using a simple linear regression model with intercept. Similarly, a linear regression model was also fitted to the type B genetic correlations over the rotation age.

Various models were tried to fit individual tree heritability, (H^2), the type B genetic correlation, r_B , and age-age correlations, r_{xy} (Lambeth 1980) using linear regressions with the parameters (H^2 , r_B , r_{xy}) as dependent variable and age as independent regressor. Using the standard estimates for H^2 and r_B , the corresponding variance components could be derived due to the standardization of the data such that the phenotypic variance was equal to one. As the single tree plot (STP) configuration is going to be used for future clonal tests, the plot error (σ^2_{hsc}) and the residual error (σ^2_e) from these row plot clonal tests were pooled together to represent the residual error (σ^2_e) of the single tree plot. These standard parameter estimates were then used in the simulation study to determine the optimal number of sites and blocks for clonal tests.

Genetic gains were estimated for mean annual increment (MAI), the trait of interest, at the time of harvesting (six years). Selection efficiency and indirect selection are of particular interest for the breeder, because they allow determination of the optimum selection age (biological optimum) at which genetic gains in the harvested crop are at maximum. This biological optimum may differ from the economical optimum (when the present value per unit of time is maximized) due to the costs that are incurred in each generation turnover (McKeand 1988, Burdon 1989).

Expected genetic gain from clonal selection for the target environment (five sites pooled) was estimated according to Falconer (1993):

$$Gy = i_y H^2_{\bar{\epsilon}_y} \sigma_{\bar{\epsilon}_y} \quad 3-6$$

where

$H^2_{\bar{\epsilon}_y}$ is broad-sense heritability based on clonal means at six years ,

i is intensity of selection at six years, and

$\sigma_{\bar{c}_y}$ is the square root of the phenotypic variance of clonal means at six years.

Broad-sense heritability of clonal means was calculated based on the clonal phenotypic standard deviation and expressed as:

$$H^2_{\bar{c}} = \frac{\sigma_c^2}{\sigma_c^2 + \frac{\sigma_{cs}^2}{s} + \frac{\sigma_e^2}{bs}} \quad 3-7$$

where

σ_c^2 , σ_{cs}^2 , and σ_e^2 are the clonal variance, clonal by site interaction variance, and residual variance respectively and

s is the number of sites, and b is the number of blocks per site.

The efficiency of early selection, or relative genetic gain, for MAI was calculated using the ratio of expected genetic gain selection at three and four years age (G_x) and maximum direct genetic gain at the same ages ($G_{\max}$), since preliminary analyses had shown these to be the best ages. Thus, relative genetic gain was calculated for a variable number of sites up to 10 sites and a maximum number of 12 blocks for each selection age.

The maximum direct genetic response at three and four years was obtained assuming an infinite number of sites and blocks and is considered the maximum possible gain achievable at that age. Efficiency of early selection is expressed as:

$$E = \frac{G_x}{G_{\max}} = \frac{i_x H^2_{\bar{c}_x} \sigma_{\bar{c}_x}}{i_y H^2_{\bar{c}_y} \sigma_{\bar{c}_y}} \quad 3-8$$

$$\text{and } E = \frac{H^2_{\bar{c}_x} \sigma_{\bar{c}_x}}{\sigma_{\bar{c}_y}} \quad 3-9$$

If it is assumed that intensity of selection is the same at an early age (i_x) and at the same age under maximum genetic gain (i_y). However, the clonal phenotypic variances ($\sigma^2_{\bar{e}_x}, \sigma^2_{\bar{e}_y}$) are distinct due to the variable number of combinations of sites and blocks in the numerator and the infinite number of those factors in the denominator which make the clonal phenotypic variance ($\sigma^2_{\bar{e}_y}$) a constant for all simulations.

Determining Optimal Selection Age

The efficiency of early selection for MAI was calculated using the ratio of indirect selection at different ages (G_x) to maximum genetic gain at six years age ($G_{\max 6}$). The maximum genetic response at six years age was obtained assuming an infinite number of sites and blocks, and is considered the maximum possible gain obtainable from selecting tested clones at rotation age for a given selection intensity. Selection efficiency was calculated for a variable number of sites up to 10 and a maximum number of 12 blocks per site for each selection age.

Genetic gain at rotation age based on an early age or based upon selection for a different trait is defined as indirect genetic gain and it is expressed as (Falconer 1993):

$$G_x = i_x H_{\bar{e}_x} H_{\bar{e}_y} r_{cy} \sigma_{\bar{e}_y} \quad 3-10$$

where

i is intensity of selection at an early age or for a different trait,

$H_{\bar{e}_x}$ is broad-sense heritability based on clonal means at an early age or for a different trait,

r_{cy} is the clonal genetic correlation between two traits or for the same trait at different ages,

$H_{\bar{c}y}$ is broad-sense heritability based on clonal means at six years, and

$\sigma_{\bar{c}y}$ is the square root of the phenotypic variance of clonal means at six years.

Selection efficiency is considered as the proportion of gain of indirect selection with respect to the gain from selection at rotation age and is estimated as:

$$E = \frac{G_x}{G_{\max 6}} = \frac{i_x H_{\bar{c}x} H_{\bar{c}y} r_{cxy} \sigma_{\bar{c}y}}{i_y H_{\bar{c}y}^2 \sigma_{\bar{c}y}} \quad 3-11$$

and

$$E = \frac{H_{\bar{c}x} H_{\bar{c}y} r_{cxy} \sigma_{\bar{c}y}}{\sigma_{\bar{c}y}} \quad 3-12$$

If it is assumed that intensities of selection are the same ($i_x = i_y$) at selection and rotation age. However, the clonal phenotypic variances ($\sigma_{\bar{c}x}, \sigma_{\bar{c}y}$) are different due to the variable number of combinations of sites and blocks in the numerator and the infinite number of those factors in the denominator.

For all calculations regarding optimal selection age, the following assumptions were made: (1) Rotation age is equal to six years, (2) Propagation time in clonal gardens including the rooting phase is two years, (3) Realization of benefits from testing and clonal selection are equivalent to selection age plus eight years (two years for bulking up in step 2 and six years after planting until harvest), and (4) Genetic parameters are stable over generations.

To determine the optimal age of selection, an analysis was conducted to maximize the discounted benefits from an infinite number of rotations of early selection. This is similar to the selection efficiency used previously (McKeand 1988, White and Hodge

1992, Balocchi *et al.* 1994) except that the new method accounts for the benefits in perpetuity.

Discounted selection efficiency (DSE) was obtained as the product of selection efficiency (E) and a discounting factor for perpetual generations (Klemperer 1996). The discounting factor (D) is based on the general formula used in forestry to estimate the present value (V_0) of perpetual periodic series of harvesting revenues (p) per acre, using a discount rate (i), at the end of every rotation of t years and expressed as (Klemperer 1996):

$$V_0 = \frac{p}{(1+i)^t - 1} \quad 3-13$$

so that, genetic gains obtained at either early age or rotation age are adjusted by the discount factor, with t equivalent to selection age plus eight years for propagation and harvesting of the material.

Discounted selection efficiency is calculated as follows:

$$DSE = E * D \quad 3-14$$

$$DSE = \frac{H_{\bar{c}x} H_{\bar{c}y} r_{cy} \sigma_{\bar{c}y}}{\sigma_{\bar{c}y}} * \frac{(1+i)^{y+8} - 1}{(1+i)^{x+8} - 1} \quad 3-15$$

where

E is defined as selection efficiency in Equation 3-12 and

D is the discounting factor

i is interest rate (5%, 10%, 15%, 20%)

x is early selection age (2, 3, 4, 5 years)

y is age at time of harvest (6 years)

and all other factors as were defined in equation 3-9.

Results and Discussion

Genetic Parameter Estimates

Age-age correlations

The genetic correlations for MAI at different ages with MAI at rotation age (MAI6) were low for Maravillas and the target environment at two years ($r_{xy} < 0.5$) but were high at all three environments at three years ($r_{xy} > 0.8$) and thereafter (Figure 3-1). Reports in the literature on age-age correlations including rotation age for clonal material of *Eucalyptus grandis* are limited to the first few years (Lambeth *et al.* 1994) and only a few are published for clonal hybrid populations (Bouvet 1991) or seedlings of *Eucalyptus* species (van Wyk 1990, Borralho *et al.* 1992b, Marques *et al.* 1996, Wei and Borralho 1998).

High age-age genetic correlations ($r_{xy} > 0.70$) for diameter (DBH) between years one and five had been reported for *Eucalyptus urophylla* in China (Wei and Borralho 1998). High genetic correlations ($r_{xy} > 0.88$) for diameter (DBH) between 2.5 years and 6.5 years were obtained for *Eucalyptus cloeziana* in Brasil. In a similar fashion, strong genetic correlations ($r_{xy} > 0.95$) were also found for sectional area at four years with sectional area at eight, 13 and 18 years for *Eucalyptus globulus* in Portugal (Borralho *et al.* 1992b). Differences between these previous studies and the present estimates of genetic correlations for MAI at two years in this study might be associated with the effect

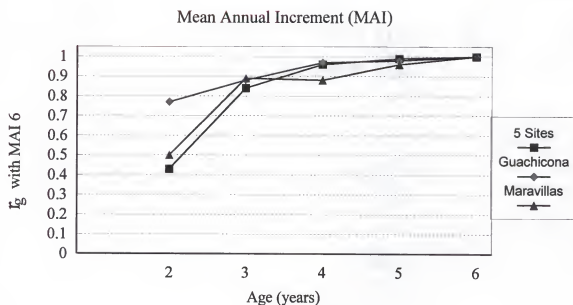


Figure 3-1. Age-age genetic correlations (r_g) for mean annual increment (MAI) at different ages with MAI at six years in clonal trials of *Eucalyptus grandis*.

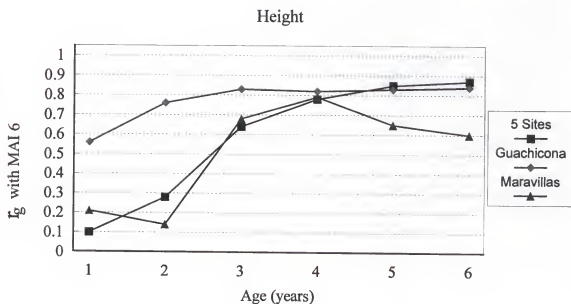


Figure 3-2. Age-age genetic correlations (r_g) for height at different ages with MAI at age 6 years in clonal trials of *Eucalyptus grandis*.

of including survival in the response variable and with the reaction of diameter growth to this effect.

The genetic correlations between height at three years (HT3) and height at six years (HT6) were high at Guachicono ($r_{xy}=0.94$) and the target environment ($r_{xy}=0.77$) but only moderate at Maravillas ($r_{xy}=0.51$) (probably due to the high incidence of broken-top damage). High age-age genetic correlations for height ($r_{xy} > 0.78$) between years one and five had been reported for *Eucalyptus urophylla* (Wei and Borralho 1998). Comparable genetic correlations for height between year 4 and years 8, and 13 in *Eucalyptus globulus* (Borralho *et al.* 1992b) were also high ($r_{xy}=0.77$) at all ages, except at year one ($r_{xy}=0.44$).

Wood density showed very high genetic correlations between three and six years age in the target environment ($r_{xy}=0.95$) and in Maravillas ($r_{xy}=0.90$) and a moderate correlation but smaller than in the previous environments in Guachicono, $r_{xy}=0.71$ (Table 3-2). Similar, genetic correlations ($r_{xy}=0.93$) between years three and six had been reported for *E. nitens* in Australia (Greaves *et al.* 1997).

Trait-trait correlations

Age-age genetic correlations between height and the target trait, mean annual increment at six years (MAI6) are presented in Figure 3-2. In general the genetic correlations tended to increase with age except at Maravillas where the age-age genetic correlation between HT and MAI6 decreased after the fourth year. At Guachicono the age-age genetic correlations started high at year one and stabilized ($r_{xy}=0.8$) from year three. In the target environment the age-age genetic correlation was low at two years

($r_{xy}=0.43$) but increased in the range of ages approaching one as the predictor trait approached the target trait.

The lower genetic correlations for height at Maravillas in years one and two could be associated with the increase in mortality between the fourth and sixth years that may have changed the frequency of ramets per clone reducing the genetic correlation with the target trait.

Genetic correlations between height and MAI at the same ages, either at three or six years, were high and positive, $r_{xy}=0.80$ to 0.87 , in the target environment and Guachicono but moderate in Maravillas, $r_{xy}=0.51$ to 0.60 (Table 3-2). This is in agreement with the genetic correlations for height from years one to six with sectional area at eight years where high ($r_{xy}=0.77$) at all ages, except at year one ($r_{xy}=0.44$) for *Eucalyptus globulus* in Portugal (Borralho *et al.* 1992c).

The genetic correlations between height and wood density either at the same or different years were almost zero between the target environment and Guachicono but moderate to low in Maravillas.

Published reports on trait-trait correlations of clonal material of *E. grandis* are very scarce and most studies on the subject are related to *E. grandis* hybrids (Bouvet and Bailleres 1995) or *E. grandis* from seedling origin (Malan 1991). These studies coincide in that genetic correlations between growth traits and wood density for *E. grandis* and its hybrids were of moderate magnitude but negative. Large negative genetic correlations between growth traits, height and diameter, and wood density were found among full-sib families of *E. grandis* in South Africa (Malan 1988).

Table 3-2. Trait – trait genetic correlations between height (HT), mean annual increment (MAI) and wood density (WD) of clonal trials *Eucalyptus grandis* in different environments at three and six years. Broad sense heritabilities are shown on the diagonals.

Trait	HT 3	HT 6	MAI 3	MAI 6	WD 3	WD 6
<u>5 Sites</u>						
HT 3	0.11					
HT 6	0.77	0.19				
MAI 3	0.80	0.75	0.14			
MAI 6	0.64	0.87	0.84	0.22		
WD 3	0.14	-0.05	-0.04	0.0	0.53	
WD 6	0.0	0.06	0.03	-0.08	0.95	0.35
<u>Guachicono</u>						
HT 3	0.46					
HT 6	0.94	0.52				
MAI 3	0.80	0.73	0.43			
MAI 6	0.83	0.60	0.89	0.52		
WD 3	0.04	0.00	-0.03	-0.06	0.34	
WD 6	-0.04	0.02	-0.07	-0.06	0.71	0.29
<u>Maravillas</u>						
HT 3	0.11					
HT 6	0.51	0.09				
MAI 3	0.80	0.19	0.21			
MAI 6	0.68	0.60	0.89	0.21		
WD 3	0.45	0.04	0.49	0.40	0.40	
WD 6	0.27	0.06	0.21	0.16	0.90	0.41

However, this study and others from *Eucalyptus* species of seedling origin indicate that the genetic correlations between growth traits and wood density are either low or nil in most cases (Borrhalho *et al.* 1992c, Greaves *et al.* 1997, Wei and Borrhalho 1997, Tibbits and Hodge 1998).

Optimal selection ages for *Eucalyptus* species, managed in a short-rotation, have been reported as 12 months for height and DBH in *E. urophylla* (Wei and Borrhalho 1998), 2.5 years for DBH in *E. cloeziana* (Marques *et al.* 1996), 3.0 years for *Eucalyptus* hybrids (Bouvet 1991) and 4.0 years on height for *E. globulus* on a rotation of eight years. In this study the higher genetic correlations of height and MAI at three years with MAI at six years versus the correlations with two years, and the small increase in broad sense heritabilities over time, would indicate that three years is the minimum biological age for selection. Furthermore, the high genetic correlations of wood density between three and six years put confidence on age three for selection on one trait or on a combination of traits based on a selection index for deployment purposes.

Standard parameter estimates

The linear regression for the modeling of individual tree heritability (H^2) using the observed values as dependent variable (Table 3-3) and age as the independent variable gave a good fit to the data ($r^2_{adj}=0.90$) indicating a significant age effect ($p=0.01$). A model to describe age trends in heritabilities for short rotation species was recently proposed (Wei and Borrhalho 1996). The model is based on increments of growth traits, and assume a genetic correlation of unity ($r_{xy}=1$) between ages an assumption not satisfied in the present study due to the low genetic correlation at two years.

Table 3-3. Standard genetic parameter estimates for mean annual increment (MAI) to be used in the simulation. The variance components σ_c^2 , σ_{cs}^2 , σ_e^2 are variances due to clonal effects, clone by environment interaction effects and residual effects respectively. Variance components are expressed as a fraction of a phenotypic variance equal to unity due to data standardization. The value H^2 is the broad-sense individual heritability (Equation 3-3), r_B is the type B genetic correlation (Equation 2-6) and r_{xy} is the genetic correlation between MAI at each age and MAI at six years (Equation 3-2).

Age	Variance Components Estimates			Standard Parameter Estimates		Observed Parameter Values		
	σ_c^2	σ_{cs}^2	σ_e^2	H^2	r_B	H^2	r_B	r_{xy}
2	0.1301	0.1074	0.7625	0.130	0.548	0.14	0.58	0.435
3	0.1518	0.1159	0.7323	0.152	0.567	0.14	0.53	0.838
4	0.1735	0.1223	0.7042	0.174	0.587	0.17	0.58	0.964
5	0.1953	0.1270	0.6777	0.195	0.606	0.20	0.61	0.992
6	0.2170	0.1300	0.6530	0.217	0.625	0.22	0.64	1

Due to the fluctuation of the type B genetic correlation from age two to age six for MAI the fit of the linear regression for this parameter was weak ($r_{adj}^2=0.41$) and the independent variable was significant at the probability level of $p=0.15$. However, for the small range of ages used the predicted values increase smoothly indicating a lower type B genetic correlation as trees get older, a more reasonable option than considering stable correlations across ages. Furthermore, the regression model is more conservative in the sense that it gives more weight to selection at later years than at early years.

Age-age correlations were estimated using the logarithmic transformation of Lambeth (1980) based on the general formula developed for several conifer species:

$$r_p = 0.308 + \log_e (\text{younger age/older age}) \quad 3-16$$

The predicted phenotypic correlation for MAI at two years with MAI at rotation age was higher ($r_p=0.68$) than the observed value ($r_{xy}=0.43$). Even if it were assumed that

the phenotypic correlation is equal to the genetic correlation Lambeth's formula, overestimates the observed value by 50%.

A simple linear regression on the observed values of the genetic correlations showed a weak fit to the data ($r^2_{adj}=0.63$) and a significant effect of age at $p\text{-value}=0.07$. However, the predicted genetic correlation for MAI between year two and six ($r_{xy}=0.59$) was not close to the observed value ($r_{xy}=0.43$). Therefore, it was decided to use the observed age-age correlations given that no appropriate models were found and the abrupt change from year two to three may be real and closer to the true values.

The standard parameter estimates used in the simulation study to determine the optimal number of sites and blocks for clonal tests are presented in Table 3-2.

Optimal Test Designs

Selection efficiencies for MAI based on direct selection at three and four years with respect to the maximum gain achievable at the same ages assuming an infinite number of sites and blocks are presented in Figure 3-3. Using a variable number of 12 ramets per clone and site, and a maximum of 10 sites in a single tree plot design, 90% selection efficiency is obtained from combinations of (1) five sites and twelve blocks, (2) six sites and six blocks (3) eight sites and four blocks and (4) ten sites and two blocks by selecting at three years age.

In all cases higher selection efficiencies are obtained by increasing the number of sites and decreasing the number of blocks. The increase in efficiency of selection is associated with the increase of the clonal mean heritability, $H^2_{\bar{\epsilon}}$ (Equation 3-9) due to the increment in the number of sites and ramets per clone.

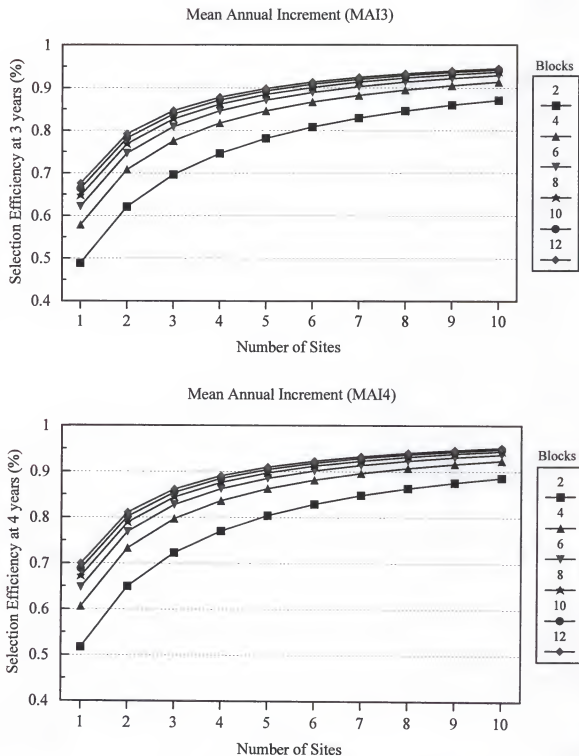


Figure 3-3. Selection efficiency for mean annual increment (MAI) of direct selection at three and four years with respect to maximum gain at the same age achievable from an infinite number of sites and blocks. Genetic parameters used to conduct these simulations were from Table 3-2.

It implies that for a pre-determined selection efficiency, i.e. 80%, a large number of clones with as few as two to four ramets per clone can be tested in a reasonable number of locations (six). It is also noted that relative increments in selection efficiencies are large when the number of blocks increase from two to six blocks but are small and almost similar when more than six blocks are used. A slightly higher similar response is obtained when selection age is at four years due to its proximity to rotation age.

Similar responses in the efficiency of progeny and clonal testing had been reported previously. An optimum number of 1 to 6 ramets per clone was found to maximize genetic gains under an scenario of multistage recurrent selection (Shaw and Hood 1985). Results of a simulation of clonal selection based on a characteristic or index with broad sense heritability less than about 0.6, indicate that two to six ramets per clone and site yield maximum gain (Russell and Libby 1986). In the same study, predictions of genetic gains using real data from *Pinus radiata* indicated that maximum gains occurred with two and three ramets per clone.

Comparable results were obtained in examining optimal tests designs for advanced generations of slash pine, under different scenarios of genetic parameters (White and Hodge 1992). In that study the use of up to 4 blocks per location was at least as efficient as use of one block per location.

All the studies reviewed pointed out that maximum gains are achievable with a low number of ramets per clone or trees per family allowing the testing of a large number of genotypes for deployment or breeding screening. However, selection of an optimal number of sites and clones for the same efficiency of selection will ultimately depend on biological considerations, political regulations and economical or logistic constraints to

carry over the program goals. For a pre-determined selection efficiency between 80 and 90% a test design spread on six locations with six blocks per location seems to be a good option. It provides an acceptable number of locations to test for genotype-by-environment interaction if present and sufficient number of ramets as a buffer for random mortality.

Optimal Selection Age

Discounted selection efficiency values (DSE) were calculated based on interest rates, 5% 10% 15% and 20%, higher than those commonly reported in the literature for this type of analyses (McKeand 1988, Balocchi *et al.* 1994). These interest rates include the risk of uncertainty of investments in the forestry business under the scenario of inflationary economies, a common scenario where this tropical species is planted.

Discounted selection efficiency based on a fixed number of six blocks per site indicated that the optimum selection age decreased with increasing interest rates (Figures 3-4 and 3-5). At 5% interest rate years discounted selection efficiency (DSE) reach its maximum at four years, being only 3% higher than DSE at three years for a variable number of locations. At the other interest rates, 10% 15% and 20%, DSE is higher at three years age and its absolute value increases as the interest rate does.

By choosing an optimum selection age as three years DSE increases about 10% for each 5% change in the interest rate. For the same age the incremental difference in DSE is higher between two and four locations, or four and six locations than for the range comprising six to twelve locations. It is also shown that differences between incremental values of DSE are smaller as the values approach rotation age.

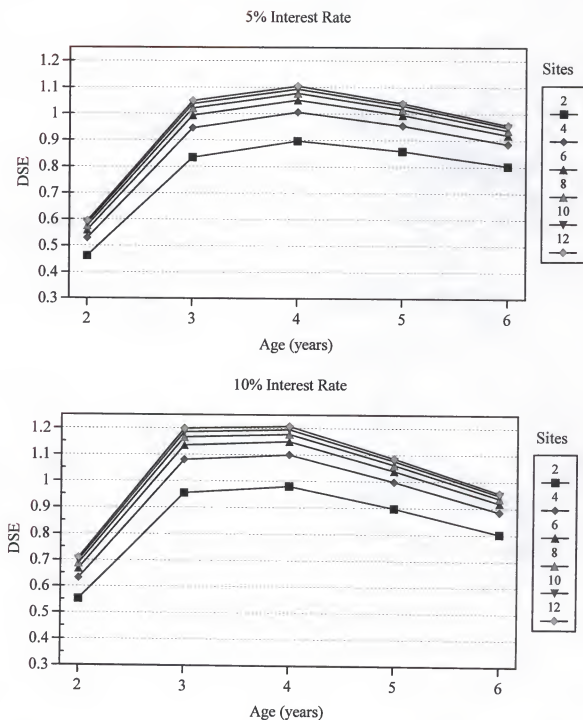


Figure 3-4. Effect of discounted selection efficiency at perpetuity (DSE from equation 3-16) on selection age using a discount rate of 5% and 10% for a randomized complete block design with single tree plots and a fixed number of six blocks from age two to six in clonal trials of *Eucalyptus grandis*. The time to realize the benefits is 14 years corresponding to a six year rotation plus eight years for propagation and harvesting.

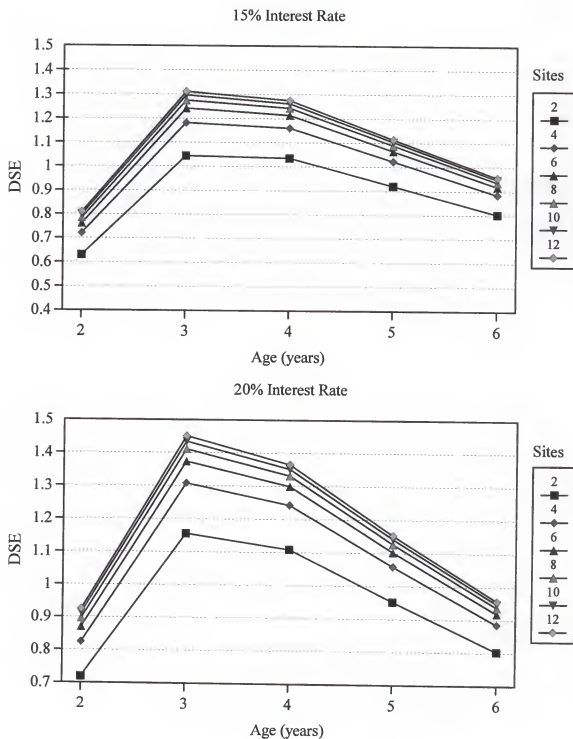


Figure 3-5. Effect of discounted selection efficiency at perpetuity (DSE from equation 3-16) on selection age using a discount rate of 15% and 20% for a randomized complete block design with single tree plots and a fixed number of six blocks from age two to six in clonal trials of *Eucalyptus grandis*. The time to realize the benefits is 14 years corresponding to a six-year rotation plus eight years for propagation and harvesting.

Comparable results were obtained for *Pinus elliottii* indicating that selection efficiency is increased either through the use of more locations (6 vs 12 locations) or by increasing the interest rate used in the discounted selection efficiency (White and Hodge 1992). Similar results were found with *Pinus taeda* where the lowest optimum selection age (six years) was for selection among full-sib families based on the total genetic component and the higher interest rates used in the study (Balocchi *et al.* 1994).

As the efficiency of indirect selection is a function of the heritabilities of the traits and the genetic correlation between them, MAI seems to be a better predictor of MAI at six years than height. Mean annual increment (MAI) had higher age-age correlations at all ages (Figures 3-1 and 3-2) and higher heritabilities showing a steadily increase from year two to six in the target environment (Figure 2-2).

Studies determining discounted selection efficiency are usually based on existing data from a single generation (McKeand 1988, Balocchi *et al.* 1994, White and Hodge 1992) or based on simulations for a few generations (Kang 1985, Newman and Williams 1991). This is probably due to the fact that turnover of generations of coniferous species limits the availability of genetic parameters over multiple generations. On the other hand, the quantitative genetic theory to estimate genetic gains over several generations of selection, taking into account changes in genetic parameters due to directional selection, has not been developed (Kang 1985, Wu 1998) leading to the debatable assumption of stable genetic parameters over time.

Even though several generations of directional selection are expected to alter the genetic variances (Bulmer 1980), this effect is expected to be counteracted by the infusion of new genes in each breeding cycle, increasing the genetic variability in the

breeding population. Furthermore, larger heritabilities and higher genetic correlations would be obtained as improved test designs are developed and clonal tests are established with better control of the environmental factors.

Although the advantages of early selection are more attractive to long rotation species (Magnussen 1989) and the precise timing of the early selection is not as critical as for other species (Borralho *et al.* 1992), its determination is still important if economical considerations are taken into account. It should be noted that in this study discounted selection efficiency (DSE) at perpetuity was compared on the basis of gain per generation by compounding each rotation's cash flows to rotation-end into one net value p (Equation 3-13). Even though the equations for DSE are simple they provide a basis for more complex models such as for "overlapping breeding generations". Under this strategy of improvement the application of DSE in perpetuity would be similar to the "soil expectation value" or "bare land value in economics" (Klemperer 1996) that allows the calculation of a net present value including gains and costs at any year.

Conclusions

The determination of age-age genetic correlations for both height and mean annual increment showed that strong age-age correlations are present for a rotation age of six years starting at three years age and thereafter, suggesting this age as appropriate for selection. This biological selection age coincides with an "economical" age for selection based on discounted selection efficiency in perpetuity, considering a range of interest rates between 5% and 20%.

High genetic correlations between wood density at three years and six years as well as the lack of genetic correlation between this trait and growth traits, also favors selection at age three and facilitates breeding strategies that consider the utilization of both traits for improvement.

A simulation of optimal test designs indicated that by using six locations and about six blocks per location in a single tree plot design between 80% and 90% of the maximum selection efficiency could be obtained. This type of design allows testing of a large number of genotypes across a reasonable number of locations in a cost effective manner.

CHAPTER 4

ESTIMATION OF GENETIC PARAMETERS FOR SEEDLING POPULATIONS OF EUCALYPTUS GRANDIS AND COMPARISONS WITH CLONAL POPULATIONS FROM ROOTED CUTTINGS

Introduction

A formal tree improvement program for *E. grandis* began in Smurfit Carton de Colombia (SCC) in 1969 and by 1982 the local land race of "Chupillauta" was producing enough seed for operational plantations. The research program at SCC has as its breeding objective to improve wood quality and timber yield at rotation age, based on volume growth and wood density as the selection criteria.

At the end of 1986, a clonal propagation program based upon rooted cuttings was initiated (Lambeth *et al.* 1989) from which 460 donor trees (clones) produced enough cuttings to be included in the first clonal tests (Lambeth *et al.* 1994). After three years in the field the best 100 clones for volume growth, straightness and other wood quality trials were recommended for further clonal testing (series 2-36) and for the establishment of a clonal seed orchard. Thus, in 1991 the seed orchard of La Arcadia was established and two years later three trials (series 2-71) containing 69 open-pollinated families were planted across three locations, in order to obtain estimates of genetic parameters and to rank the best parents for the operational program.

In 1995 four genetic tests (series 2-73) comprising 238 open-pollinated families from the local land races, the natural range, and from exchange with different forestry organizations were established to serve as a base population for the next generation of breeding. Infusions from external sources are aimed at improving the traits of interest for the program and to increase the genetic variability in the breeding population.

Obtaining accurate and precise genetic parameter estimates (heritabilities, genetic correlations between different traits, genotype-by-environment interaction and variance ratios) is fundamental to determining breeding strategies and choosing genotypes for commercial propagation (White 1987). Precise parameter estimates are also essential in the prediction of breeding values and genetic gains when the methods of ranking genotypes are selection index (SI), best linear prediction (BLP) or best linear unbiased prediction (BLUP) (White and Hodge 1989; White 1996).

In some tree improvement programs using clonal multiplication, trees are selected based on their performance in seedling progeny tests and subsequently included in the vegetative propagation program. This practice leads to reduced genetic gains if the genetic correlation between trees from seedling origin and vegetative propagules (clones) is not favorable and strong (Burdon *et al.* 1992, Frampton and Foster 1993). Comparisons between rankings of clones and open-pollinated seedlings from the same trees for *Eucalyptus grandis* varied from low (Oda *et al.* 1989, Ikemori 1990) to moderate (Kageyama and Kikuti 1989). Nevertheless, comparisons between seedlings and clones taken from other seedlings of the same family for *Eucalyptus grandis* (Ikemori 1990),

Picea abies (Skroppa and Dietrichson 1989), *Pinus taeda* (Frampton and Foster 1993) and *P. radiata* (Burdon *et al.* 1992) have been generally higher.

The objectives of this chapter are subdivided into two main parts. First, data from two distinct series of open-pollinated seedling tests are used to: (1) evaluate the contribution of new infusions to the breeding population of Smurfit Carton de Colombia (SCC), and (2) estimate genetic parameters from these tests that will be used to build strategies for the seedling phase of the SCC tree improvement program. Second, the data from the seedling trials were combined with that from five clonal trials established with rooted cuttings from 44 of the seed orchard parents (clones) whose offspring were planted in the seedling trials. The goal is to assess the genetic correlation between: (1) breeding values of the parents based on performance of their seedling offspring and (2) clonal genetic values based on the performance of their rooted cuttings.

Materials and Methods

The location and climatic conditions of the two series of seedling genetic tests are presented in Table 4-1. These sites are representative of the target environment for plantations of *Eucalyptus grandis* in SCC.

The progeny tests of series 2-71 had the dual objectives of testing the genetic value of the seed orchard parents for roguing and improvement of seed quality for deployment, as well as providing information on genetic parameters of the species. Series 2-73 was established as part of the breeding strategy of *E. grandis* with the multiple objectives of establishing a broad genetic base of 238 Open-pollinated families,

estimating genetic parameters, and providing the base population for the next generation of breeding.

Table 4-1. Location and climatic conditions of seedling tests of *Eucalyptus grandis* corresponding to series 2-71 and 2-73 in Smurfit Carton de Colombia.

Series	Location	Test	Latitude (N)	Longitude (O)	Altitude (m)	Mean Annual Rainfall (mm)	Mean Annual Temperature (°c)
2-71	Chupillauta	1	2° 29'	76° 40'	1750	2155	18.3
	Samaria	2	3° 53'	76° 27'	1750	2500	17.7
	Indostan	3	4° 15'	75° 47'	1740	2414	18.3
2-73	Aguaclara	4	3° 41'	76° 33'	1500	920	16.0
	El Riel	5	4° 51'	75° 33'	1680	2822	17.1
	San Jose	6	2° 36'	76° 34'	1750	2050	18.5
	Suiza	7	3° 49'	76° 28'	1560	1156	18.9

Seed collection for series 2-71 was carried out in the Arcadia clonal seed orchard of *E. grandis*. The seed orchard was established in December 1991 and it was 2.5 years old when the seed for study 2-71 was collected. Open-pollinated seed for series 2-73 was obtained from nine different genetic groups representing different stages of improvement of the local land race by the SCC tree improvement program, land races 1 to 3 (LR1, LR2, LR3), the natural range of the species (NR), and tree improvement programs from five forestry organizations in Brazil and South Africa (EX1 to EX5). Each source or genetic group was represented by from 11 to 73 open-pollinated families and details of each group are in Table 4-2.

Site preparation and cultural practices were basically the same at all sites in both series and followed the standard practices applied by the operational program. The experimental design in both series was a randomized complete block design (RCBD). In

series 2-71, there were seven blocks per site planted in six-tree row plots with 67 to 69 families per site established by the end of 1993. In series 2-73, there were 12 to 25 blocks per site planted in single tree plots in May 1995.

Table 4-2. Genetic groups of *Eucalyptus grandis* and their corresponding families tested in series 2-73 across four sites.

Genetic Group	Code	Provenance / Seed Source	Families (No)
First plantations at SCC.	LR1	Originated from three other land races and seed from Zimbabwe.	14
Seed orchard of "La Arcadia"	LR2	The best 100 selections for the clonal program from the first plantations	73
Seed production area of "Chupillauta"	LR3	Originally a provenance trial with 5 provenances and 20 seed sources. Ten more selections from plantations were added	35
Selected trees from the natural range	NR	Five provenances represented by five families per provenance	25
Exchange seed from five forestry organizations	EX1 to EX5	Seed from other breeding programs. Four organizations represented by 20 families and one by 11 families	91

Data Collection

Survival (%), total height (m) and diameter at breast height (cm), DBH, were measured at age three years. Also at age three, basic wood density was sampled by randomly selecting three trees from each family in series 2-71 in each block and extracting, at breast height, 5mm wood increment cores. In study 2-73 wood density was sampled by choosing at random ten blocks in each site and taking wood cores of all trees in the block. Basic wood density was estimated by the water volume displacement method (ASTM 1969). Individual tree volume and mean annual increment (MAI) were calculated for each at three years age. Individual tree volume, overbark, determinations were made according to the following volume equation developed for seedlings by the Planning Department of Smurfit Carton de Colombia (Uribe 1995):

$$\text{Volume (m}^3\text{)} = 0.014 + 0.318D^2H \quad 4-1$$

For series 2-71, with row plot configuration, mean annual increment (MAI) was estimated by multiplying each tree volume by the corresponding plot survival and expressing it on a mean annual basis per unit of area ($\text{m}^3 \text{ ha}^{-1} \text{ yr}^{-1}$). In series, 2-73 due to its configuration in single tree plots, each tree volume was multiplied by the survival of the family in the site and expressed on a mean annual basis ($\text{m}^3 \text{ ha}^{-1} \text{ yr}^{-1}$).

Data Editing and Standardization

Prior to analyses, editing of the data was performed to remove recording errors and dwarf trees that were potentially inbred trees. The identification of potential outliers was conducted by building a linear regression for height and diameter at each block in

each site using PROC INSIGHT (SAS® 1993). In all, fewer than 3% of the observations per block were deleted from the data based not only on their statistical information, but also on the physical and biological observations supplied at the time of measurements.

The analyses to estimate differences among genetic groups were performed on untransformed data since no trend was perceived between the error variance and the mean of each trait across tests. However, before the analyses to estimate genetic parameters the square root of the block phenotypic variance was used to standardize all continuous traits to create homogeneous variance structures across tests (Falconer 1993, White 1996). That is, for each growth trait and wood density, each tree measurement was divided by the phenotypic standard deviation of its corresponding block producing a transformed variable with phenotypic variance of one.

Genetic Group Comparisons

The genetic tests of series 2-73 consisted of nine genetic groups whose assigned codes and number of families per group are shown in Table 4-2. These groups sampled the genetic variability and potential performance present in the natural range of the species (NR), from different levels of improvement of the local land race at SCC (LR1 to LR3) and other forestry organizations that use *Eucalyptus grandis* as one of their main species for commercial forestry (EX1 to EX5).

Pooled analyses of variance combining data across all four sites were performed on survival, height, individual tree volume and mean annual increment (MAI) at three years age. The analyses were conducted using PROC GLM (SAS® 1990) to obtain the

“F” tests for the random effects (with Satterthwaite option) and with PROC MIXED (Littell *et al.* 1996) to test for the fixed effects. The analyses were based on individual tree data for all growth traits and survival (0/1 data) according to the following linear model:

$$Y_{ijkl} = \mu + s_i + b_{j(i)} + G_k + sg_{ik} + bg_{ijk} + e_{ijkl}$$

where

Y_{ijkl} is the observable trait for each individual tree;

μ is the general mean;

s_i is the random effect of the i^{th} site, with $i = 1, \dots, 4$ and $s_i \sim N(0, \sigma_s^2)$;

$b_{j(i)}$ is the random effect of the j^{th} block in the i^{th} site, with $j = 1, \dots, 25$;

G_k is the fixed effect of the k^{th} genetic group, with $k = 1, \dots, 9$;

sg_{ik} is the random interaction effect between the i^{th} site and the k^{th} group,

with $sg_{ik} \sim N(0, \sigma_{sg}^2)$;

bg_{ijk} is the random effect of the k^{th} group with the j^{th} block, and i^{th} site,

with $bg_{ijk} \sim N(0, \sigma_{bg}^2)$; and

e_{ijkl} is the random residual effect of the i^{th} individual of the ijk^{th} plot, with

$e_{ijkl} \sim N(0, \sigma_e^2)$

Least square means (LSM) for the nine groups were obtained for all traits from PROC MIXED and differences among groups were compared by using single degree-of-freedom contrasts. In general, survival was high for all sites (about 90%) and no further analyses were conducted on this trait. The trait of interest is MAI. Therefore, additional

examination of the group by site interaction of mean annual increment (MAI) was performed by graphical analyses.

Estimates of Genetic Parameters from Seedling Tests

Overall estimates of genetic parameters such as heritabilities and type B genetic correlations (r_B) were made from seedling trials (series 2-71 and 2-73). Estimates of variance components for total height (m), individual tree volume (m^3), MAI ($m^3ha^{-1}year^{-1}$) and wood density (gcm^{-3}), were obtained from the multivariate computer program MTDFREML (Boldman *et al.* 1993).

The standardized data from the two seedling series were combined into a single MTDFREML run for each trait to allow comparisons among the series. The two series were treated as different traits in the bivariate application of MTDFREML. This allows different linear models to be specified for series 2-71 (row plots) and 2-73 (single tree plots).

The statistical analysis for each variable in the seedling genetic tests followed the bivariate mixed model, in matrix form:

$$\begin{bmatrix} y_1 & - \\ - & y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \begin{bmatrix} b_1 & - \\ - & b_2 \end{bmatrix} + \begin{bmatrix} 0 & Z_1 & 0 \\ 0 & 0 & Z_2 \end{bmatrix} \begin{bmatrix} a_{1,1} & a_{1,2} \\ a_{p,1} & a_{p,2} \\ \hline a_{p+1,1} & - \\ a_{p+N1,1} & - \\ \hline - & a_{p+N1+1,2} \\ - & a_{p+N1+N2,2} \end{bmatrix}$$

$$+ \begin{bmatrix} N_1 & 0 \\ 0 & N_2 \end{bmatrix} \begin{bmatrix} d_1 & - \\ - & d_2 \end{bmatrix} + \begin{bmatrix} Q_1 & - \\ 0 & - \end{bmatrix} \begin{bmatrix} p_1 & - \\ 0 & - \end{bmatrix} + \begin{bmatrix} e_1 & - \\ - & e_2 \end{bmatrix} \quad 4-2$$

where

y_1 = vector of observations corresponding to series 2-71, $i = 1$;

y_2 = vector of observations corresponding to series 2-73, $i = 2$;

b = matrix of fixed effects for site, block and group effects for series i ($i = 1, 2$);

a_i = vectors of random additive effects corresponding to traits 1, and 2, and

a_i is $MVN \sim (0, G)$; a_{i1} to $a_{p,i}$ are parental additive effects which are not directly observed;

d = vector of the uncorrelated random effects corresponding to additive by site interactions, and d is $MVN \sim (0, D)$;

p = vector of the uncorrelated random effects corresponding to block within site by additive interactions in series 2-71 (row-plot error) and p is $MVN \sim (0, K)$;

e = vectors of random residual effects for traits 1 and 2, and e is $MVN \sim (0, R)$ and

X , Z , N , and Q are incidence matrices relating records to the fixed effects, and additive by site, additive by block within site and residual effects, respectively.

The variance and covariance components are:

$$\text{Var} \begin{bmatrix} a_{1,1} & a_{1,2} \\ a_{p,1} & a_{p,2} \\ \text{-----} & \text{-----} \\ a_{p+1,1} & - \\ a_{p+N1,1} & - \\ \text{-----} & \text{-----} \\ - & a_{p+N1+1,2} \\ - & a_{p+N1+N2,2} \end{bmatrix} = G$$

where

$$\text{Var} \begin{bmatrix} a_{p,1} & a_{p,2} \end{bmatrix} = \begin{bmatrix} \sigma_{a1}^2 & \sigma_{a12} \\ \sigma_{a21} & \sigma_{a2}^2 \end{bmatrix} \text{ is the variance-covariance matrix for common}$$

parents in both traits and

$$\text{Var} \begin{bmatrix} a_{p+1,1} & a_{p+1,2} \end{bmatrix} = \begin{bmatrix} \sigma_{a1}^2 & 0.25\sigma_{a12} \\ 0.25\sigma_{a21} & \sigma_{a2}^2 \end{bmatrix} \text{ is the variance-covariance matrix for}$$

the progeny with common parents in both traits and

$$\text{Var} \begin{bmatrix} a_{p+i,1} & a_{p+i,2} \end{bmatrix} = \begin{bmatrix} \sigma_{a1}^2 & 0 \\ 0 & \sigma_{a2}^2 \end{bmatrix} \text{ is the variance-covariance matrix for the}$$

progeny without common parents.

$$\text{Var} \begin{bmatrix} d_1 & - \\ - & d_2 \end{bmatrix} = \begin{bmatrix} \sigma_{as1}^2 I_{N1as} & 0 \\ 0 & \sigma_{as2}^2 I_{N2as} \end{bmatrix} = D$$

$$\text{Var} \begin{bmatrix} p_1 & - \\ 0 & - \end{bmatrix} = \begin{bmatrix} \sigma_{abs1}^2 I_{N1abs} & - \\ 0 & - \end{bmatrix} = K$$

$$\text{Var} \begin{bmatrix} e_1 & - \\ - & e_2 \end{bmatrix} = \begin{bmatrix} \sigma_{e1}^2 I_{N1} & 0 \\ 0 & \sigma_{e2}^2 I_{N2} \end{bmatrix} = R$$

$$\text{Var} \begin{bmatrix} y_1 & - \\ - & y_2 \end{bmatrix} = ZGZ' + N DN' + Q KQ' + R$$

where

I is an identity matrix with dimension equal to the number of levels for the random effect,

σ_{a1}^2 , σ_{a12} , σ_{as}^2 , σ_{abs}^2 , σ_e^2 are the additive variance for trait 1, the covariance of additive effects between trait 1 and trait 2, the variances of additive by site, additive by block within site, and residual effects respectively.

Additional to variance components, MTDFREML provides estimates of genetic and phenotypic correlations for both series according to the following equation:

$$r_{xy} = \frac{\sigma_{xy}}{\sqrt{\sigma_x^2 \sigma_y^2}} \quad 4-3$$

where

r_{xy} is the additive or phenotypic correlation in series 2-71 and series 2-73

σ_{xy} is the additive genetic covariance or phenotypic covariance between the two series and,

σ_x^2, σ_y^2 are the additive genetic or phenotypic variances of series 2-71 and series 2-73.

Narrow-sense individual heritability for series 2-71 and 2-73 was calculated with the estimates of variance components from MTDFREML as follows:

$$h_{ns}^2 = \frac{3.3\sigma_f^2}{\sigma_p^2} \quad 4-4$$

where

σ_f^2 is the family variance and represents 1/3 of the additive variance (σ_a^2) and σ_p^2 is the existing phenotypic variance in the trait.

In open-pollinated progeny tests siblings are sometimes considered to be true half-sibs families having a coefficient of relatedness of $r=0.25$. This assumes seed collected from unrelated trees and crosses based on a large number of male parents, but in reality open-pollinated seed consists of a mixture of outcrossed and inbred seed resulting from selfing and from mating of related parents that cause the coefficient of relationship

among the progeny to be higher than expected $r=0.25$ (Borrahlo 1995). Estimates of genetic parameters for the open-pollinated tests of series 2-71 and series 2-73 are based on a coefficient of relationship of $r=0.33$, which assumes an outcrossing rate of 90% and has become an standard for *Eucalyptus* and other species in the literature.

Comparison of Seedling and Clonal Performance

In order to determine the genetic correlation in growth traits and wood density between rooted cuttings of the seed orchard clones, series 2-36, previously analyzed in Chapters 2 and 3 and their progeny in series 2-71 and 2-73, the seedling series were pooled into one data set with the clonal data. Thus a bivariate analyses was performed on a reduced data set consisting of 44 common seed orchard clones represented by their progeny in the seedling trials and as rooted cuttings in the clonal trials.

In the bivariate analysis, all data from the seven clonal trials were considered as trait 1, while the seedling data was the second trait. The statistical model for the analysis of each variable is presented in equation 4-5 and all terms related to the plot effect (σ^2_{bsf}) in trait 1 were dropped. The bivariate mixed model, in matrix form:

$$\begin{bmatrix} y_1 & - \\ - & y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \begin{bmatrix} b_1 & - \\ - & b_2 \end{bmatrix} + \begin{bmatrix} 0 & Z_1 & 0 \\ 0 & 0 & Z_2 \end{bmatrix} \begin{bmatrix} a_{1,1} & a_{1,2} \\ a_{p,1} & a_{p,2} \\ c_{1,1} & - \\ c_{p,1} & - \\ - & a_{p+1,2} \\ - & a_{p+N,2} \end{bmatrix} \\ + \begin{bmatrix} N_1 & 0 \\ 0 & N_2 \end{bmatrix} \begin{bmatrix} d_1 & - \\ - & d_2 \end{bmatrix} + \begin{bmatrix} e_1 & - \\ - & e_2 \end{bmatrix} \quad 4-5$$

where

y_1 = vector of observations corresponding to clonal series 2-36, trait 1; y_1 is missing for series 2-71 and 2-73;

y_2 = vector of observations corresponding to seedlings series 2-71 and 2-73, trait 2

b = matrix of fixed effects for site, block and group effects for traits 1 and 2 ;

$\begin{bmatrix} a_{1,1} & a_{1,2} \\ a_{p1,1} & a_{p1,2} \end{bmatrix}$ = sub-matrix of additive effects between clones and seedlings not

directly observed;

c_1 = vector of random clonal effects corresponding to trait 1, and c_1 is $N\sim(0,C)$.

There are p clones corresponding to equal number of parents.

a_2 = vector of random additive effects corresponding to trait 2, and a_2 is $N\sim(0, A)$;

d = matrix of the uncorrelated random effects corresponding to additive by site interactions, and d is $MVN\sim(0,D)$;

e = matrix of random residual effects for traits 1 and 2, and e is $MVN\sim(0,R)$ and

X , Z , N , are incidence matrices relating records to the fixed effects, clonal and additive effects, additive by site and random residual effects, respectively.

The variance-covariance matrix of random genetic effects contains the clonal variance-covariance matrix, the parental additive variance-covariance matrix, the progeny additive variance-covariance matrix and the variance-covariance matrix of additive effects between clones and progeny. The covariance between clones and progeny of the same clone can be expressed as $\text{cov}(c_{1,1}, a_{1,2}) = \text{cov}(a_{1,1} + d_{1,1} + e_{1,1}, a_{1,2}) = \sigma_{a1,2}$, and

$a_{1,i}, d_{1,i}, e_{1,i}$ represent additive, dominance and epistatic effects respectively.

The variance-covariance matrix of the uncorrelated and residual random effects:

$$\text{Var} \begin{bmatrix} d_1 & - \\ - & d_2 \end{bmatrix} = \begin{bmatrix} \sigma^2_{as1} I_{N1as} & 0 \\ 0 & \sigma^2_{as2} I_{N2as} \end{bmatrix} = D$$

$$\text{Var} \begin{bmatrix} e_1 & - \\ - & e_2 \end{bmatrix} = \begin{bmatrix} \sigma^2_{e1} I_{N1} & 0 \\ 0 & \sigma^2_{e2} I_{N2} \end{bmatrix} = R$$

$$\text{Var} \begin{bmatrix} y_1 & - \\ - & y_2 \end{bmatrix} = ZGZ' + N DN' + Q KQ' + R = Z$$

where

I is an identity matrix with dimension equal to the number of levels for the random effect,

$\sigma^2_{a1}, \sigma_{a12}, \sigma^2_{as}, \sigma^2_{abs}, \sigma^2_e$ are the additive variance for trait 1, the covariance of additive effects between trait 1 and trait 2, the variances of additive by site, additive by block within site, and residual effects respectively.

Results and Discussion

Genetic Group Differences

The analyses of survival, height, individual tree volume and mean annual increment at three years age indicated that there were significant differences ($p < 0.05$) for

all main effects, site blocks and groups, and in the interaction term site by group. The analyses for height and MAI are presented in Table 4-3.

Table 4-3. Analysis of variance for height (HT) and mean annual increment (MAI) pooled across four sites at three years of age.

Source	D.F.	HT		MAI	
		F Value	Pr > F	F Value	Pr > F
Site	3	24.97	0.0001	16.41	0.0001
Block(Site)	74	25.00	0.0001	14.03	0.0001
Group	8	24.49	0.0001	25.07	0.0001
Site*Group	24	4.14	0.0001	3.76	0.0001
Group*Block(Site)	592	0.86	N.S. ^a	0.93	N.S.

^a NS: non-significant effect at probability value of $p=0.5$

Even though there are statistically significant differences among the groups the site-by-group interaction effect seems to be unimportant in practice. The graphical method (Allard and Bradshaw 1964) showed two "clusters" of groups where only a few changes of rankings occur (Figure 4-1). One of those clusters is represented by most of the infusions from other programs (EX2 to EX5) and the seed production area of Chupillauta (LR3) and the other group from seed of the natural range (NR) and two other land races from SCC (LR1, LR2).

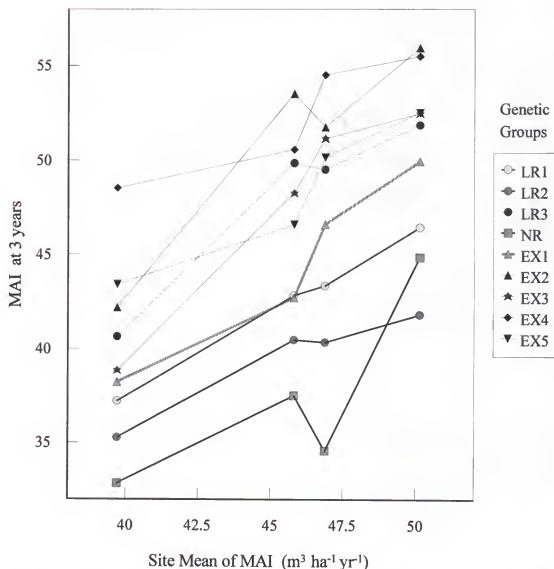


Figure 4-1. Average performance in mean annual increment (MAI) for nine genetic groups in series 2-73. These genetic groups include three land races (LR1, LR2, LR3) representing different levels of improvement at SCC, the natural range of *Eucalyptus grandis* (NR) and infusions from five forestry organizations (EX1-EX5).

The analyses of contrasts for survival (Figure 4-2) showed that seed from the natural range (NR), from the clonal seed orchard Arcadia (LR2), and from the original plantations at SCC had better survival than seed from the Chupillauta seed production area (LR3) or most of the exchange organizations (EX2 TO EX5). This indicates the variation in adaptability of the species to new environments and the capture of this trait throughout the development of a land race and subsequent selection of the individuals better fitted to the environment.

The analyses of contrasts for height and MAI showed similar results (Figures 4-2 and 4-3). On the overall, four of the five organizations that provided seed and the seed orchard of Chupillauta had a greater mean annual increment than that of the natural range or LR1 and LR2 from SCC. This result may be an indication of higher selection intensity and level of improvement of those organizations compared to SCC. The same is also true for the seed production area of Chupillauta that is constituted by 20 seed sources provided by the United States Service of Agriculture (USDA) and only 5 different provenances. Another possibility relates to the lack of a desirable source to start an expanding forestry program (Zobel and Talbert 1984). As of 1987, when the clonal program started 50-70% of the seed planted in the nursery between 1980 and 1986 came from a local landrace that may be of lower quality. This could explain the gains that had been obtained in the clonal program (LR2) and the potential that exists to obtain greater genetic gains as new infusions of improved material are brought into the program. As of 1987 the planting area developed from the seed production area of Chupillauta was proportionally less as it was recorded in the nursery. Even though this seed (LR3) performs well it has shown to be

very variable and more susceptible to diseases than the selected trees (LR2) voiding its use in large scale.

Genetic Parameter Estimates of Seedlings and Clones

Genetic parameter estimates from the seedling genetic tests, series 2-71 and 2-73 are presented in Table 4-4. Estimates from the clonal trials, series 2-36, are also included for comparison.

Table 4-4. Narrow-sense heritabilities and type B genetic correlations for height (HT), individual tree volume (VOL), mean annual increment (MAI) and wood density (WD) from two series of seedling genetic trials and one series of clonal trials of *Eucalyptus grandis*. For the seedling trials parameter estimates were derived from bivariate analyses. Those for the clonal trials were derived from multivariate analyses including two extreme environments (see Chapter 2).

Series	Type of Propagule	HT		MAI		VOL		WD	
		^a h^2_{ns} or	r_B	h^2_{ns} or	r_B	h^2_{ns} or	r_B	h^2_{ns} or	r_B
		^b H^2		H^2		H^2		H^2	
2-71	Seedling	0.21	0.72	0.30	0.86	0.27	0.77	0.63	1.0
2-73	Seedling	0.24	0.85	0.33	0.79	0.26	0.88	0.43	1.0
2-36	Cutting	0.11	0.52	0.14	0.53	0.15	0.60	0.53	0.91

^a Narrow-sense heritability for seedlings

^b Broad-sense heritability for clones

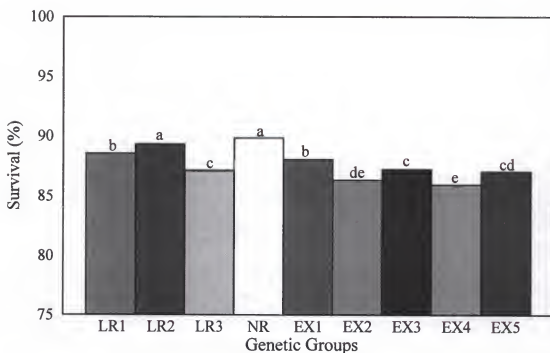


Figure 4-2. Average survival (%) of nine genetic groups in series 2-73 at three years of age. These genetic groups include three land races (LR1, LR2, LR3) representing different levels of improvement at SCC, the natural range of *Eucalyptus grandis* (NR) and infusions from five forestry organizations (EX1-EX5). Group means with same letter are not significantly different at $p = 0.05$.

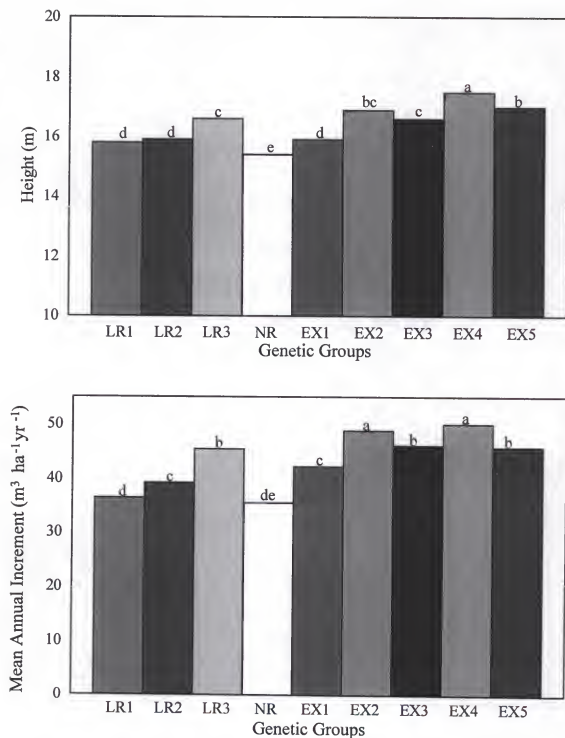


Figure 4-3. Average total height (m) and mean annual increment, MAI, ($\text{m}^3 \text{ ha}^{-1} \text{ y}^{-1}$) of five genetic groups in series 2-73 at three years of age. These genetic groups include three land races (LR1, LR2, LR3) representing different levels of improvement at SCC, the natural range of *Eucalyptus grandis* (NR) and infusions from five different organizations (EX1-EX5). Group means with same letter are not significantly different at $p = 0.05$.

In general, the results from the two series of seedling trials consistently indicate a moderate genetic control of growth traits and wood density as well as a low genotype-by-environment interaction for all traits. Narrow-sense heritabilities from these trials are larger than broad-sense heritabilities of the clonal trials for all traits. This trend of larger genetic variation for the open-pollinated progenies than for the clones may indicate that there is very low additive variance in the material represented in the clonal trials. However, as the genetic structure of the seedling and clonal series was very different the results from the modified analysis using only common progenies and clones across series may be more appropriate.

The bivariate analysis of MAI for the two series of seedlings showed that the plot error variance (σ^2_{bst}) plus the within plot variance (σ^2_e) in series 2-71 was of similar magnitude (0.65) that the plot error variance (σ^2_{st}) in the 2-73 series (0.50). Therefore the two seedling series were combined in one data set and compared to the clonal series 2-36.

The results from the bivariate analysis for growth traits and wood density including progeny and rooted cuttings from 44 common ortets are shown in Table 4-5.

These results confirm those presented in Table 4-4 showing narrow sense heritabilities larger than broad sense heritabilities for all traits and low genetic correlations between the two types of propagation methods. The individual heritabilities were verified by univariate analyses in MTDFREML to refute the possibility of errors in the specification of the bivariate analyses, and they confirm the lower genetic variability in the clonal series in comparison to the seedling series. For MAI the coefficient of

genetic variation, ($CV_g=0.33\sigma_g^2/\text{mean}$), for the progeny was larger ($CV_g=0.19$) than that from the clones ($CV_g=0.15$) also indicating lower genetic variation in the clones.

Table 4-5. Narrow-sense heritabilities and type B genetic correlations for height (HT), individual tree volume (VOL), mean annual increment (MAI) and wood density (WD) from two series of seedling genetic trials and one series of clonal trials with 44 common ortets of *Eucalyptus grandis*.

Series	Type of propagule	HT	MAI	VOL	WD
2-71 and 2-73	Seedling	0.32	0.44	0.33	0.66
2-36	Cutting	0.14	0.17	0.18	0.53
	r_g^a	0.03	0.16	0.16	0.77

^a Genetic correlation between seedlings and cuttings from the same ortet.

The available estimates of genetic correlations from different studies in *E. grandis* in Brazil are in agreement with these results. Estimates were made for growth traits at one year of age in two different sites and the results showed genetic correlations (r_g) between -0.32 and 0.33 for height and between -0.31 and 0.18 for DBH (Ikemori 1990). A higher phenotypic correlation ($r_p=0.67$) at the level of progeny means was reported by Kageyama and Kikuti (1989) in a similar type of trial. Phenotypic correlations between clones at two years and progeny means at 3.5 years of *E. grandis* were also reported for DBH ($r_p=0.02$) and height ($r_p=0.33$) in Brazil (Oda *et al.* 1989). As in this study higher narrow-sense heritability for the progeny than the broad-sense heritability for clones was

observed in some instances in the previous studies (Kageyama and Kikuti 1989, Ikemori 1990).

Lack of correlation between field performance of vegetative propagules and seedlings has been attributed to (1) propagation effects (2) nonadditive genetic variance and (3) differences in the accuracy of testing of seedlings and clones (Oda *et al.* 1989, Ikemori 1990, Borralho and Kanowski 1995). In this context, some possible explanations of the low genetic correlation between progeny and cuttings of the same ortet are proposed.

Although a coefficient of relationship of $r=0.33$ was assumed to estimate narrow sense heritability it is possible that did not account for the level of relatedness in the seedling population which could have been large if only a few parents contributed to pollination in the Arcadia seed orchard when it was 2.5 years old. In consequence variance components, additive genetic variance, within-plot variance, and individual heritabilities could be inflated (Sorensen and White 1988; Hodge *et al.* 1996). Furthermore, the evidence from field data suggests that heritabilities at young ages are inflated beyond that predicted by models (Hodge *et al.* 1996).

Adjustments of the coefficient of relationship were suggested by Squillace (1974); however, determination of the appropriate factor by this procedure is difficult and not possible under selfing rates varying across families and the presence of dominance effects (Borralho 1994). The accumulation of dominance effects as a response to high selection intensities has been also advocated to explain the lack of correlation between progenies and clones (Oda *et al.* 1989). It has been shown that the presence of dominance effects

and variable inbreeding depression within families increased bias upward in heritability estimates, and considerably reduced the genetic correlation between vegetatively propagules and seedling progeny of the same parents (Borralho 1995). Even though high genetic gains are obtained by selecting the top 25% to 30% clones of the 65 tested clones, the overall clonal variation in this population might be small due to the high intensity of selection applied i.e 1: 30770. This would also explain the low broad sense heritability of the clones compared to the heritability of the seedlings.

In a simulation study Borralho and Kanowski (1995) have also showed that in the presence of common environmental effects to some clones, "C effects" (Libby and Jund 1962), the lack of correlation becomes large and significant as the number of progeny and clones tested increased. Even though the operational production of *Eucalyptus grandis* by rooted cuttings has been done for many years the biological, genetic and physiological factors that control the sprouting and rooting processes of the cuttings are still unknown (Ikemori 1990). Furthermore, clonal differences in rooting and early growth often result in clonal differences in planting size which are one form of "C" effects (Frampton and Foster 1993).

The low genetic correlation for growth traits between seedlings and clones might also be due to carry-over effects ("C" effects) from clonal gardens. These clonal multiplication areas are established on gentle hills of different slopes and consist of unrandomized blocks of variable size per clone depending on the degree of sproutability and rootability of the clone. Besides, the collection of sprouts from each clone is made at different times, i.e. 50 to 65 days after cutting the stump, according to differences in

height, diameter and lignification of the sprout to produce cuttings with the empirical quality required for an appropriate level of rooting. Thus, some clones may have different pre-conditioning effects associated with these differential hedge conditions or harvest schedules.

The lower genotype-by-environment interaction of height at two years age in the clonal material and its increment in the following years may also indicate that some propagation effects are affecting the growth of clones in the first few years.

Maturation effects have shown strong influences in the field performance of vegetative propagules of conifer species; however, the coppicing ability of *Eucalyptus grandis* is considered to provide juvenile cuttings from meristems, axillary buds, that normally are perennially dormant and therefore mature at a slower rate than active ones (Bonga and von Aderkas 1990, Frampton and Foster 1990). Nevertheless, some degree of maturation of the clonal ortets has been previously suggested to explain differences between cuttings and seedling performance (Lambeth *et al.* 1994). It may be that after three or four cycles of propagation some maturation effects have appeared decreasing the genetic correlation between clones and seedlings.

The statistical designs, quality of lay out and the silvicultural practices conducted in all series of tests are the very similar. The coefficients of environmental variation, $CV_e = (\sigma^2_e - 0.33\sigma^2_a) / \text{mean}$, of MAI for the progeny were larger ($CV_e = 0.24$) than that from the clones ($CV_e = 0.15$), indicating more homogeneity within clones than in the progeny trials. This rules out the effect of better testing methods of the seedling trials, decreasing

the phenotypic variance, as an explanation for the larger heritability in the seedling trials and the low correlation between propagules and progeny from the same trees.

Conclusions

The evaluation at three years age of two series of seedling trials and their comparison with a series of clonal tests of the same age allowed the following statements: First, the seedling series 2-73 showed the importance and growth potential that can be achieved by the infusion of new genetic material from external sources to the breeding population. The results also indicate that the new infusions are quite stable across sites and only minor changes in ranking are present when planted as seedlings.

The higher heritability for growth traits in the progeny trials and lack of genetic correlation between the seedling and the clones might be related to several factors including: (1) overestimation of the genetic control in the seedling population due to unaccounted inbreeding, (2) existence of dominance effects in the clonal population due to high intensity of selection; (3) the presence of "C" effects in the first few years of growth; and (4) maturation effects associated with the serial propagation of the cuttings.

Although some explanations for the low genetic correlations have been attempted it should be kept in mind that the seedling and clonal series tests were established in different years and on sites which may confound the estimates and increase the error of estimation. Furthermore, the units of comparison (σ^2_s , σ^2_c) between seedlings and clones are genetically different and depending on the genetic control of the trait (i.e. growth

traits versus wood traits) the expectation of the genetic rank correlations among propagule types may be low (Frampton and Foster 1993).

For SCC, which is committed to clonal forestry the present results suggest clonal testing of all promising individuals prior to operational deployment. More information regarding the amount of additive and nonadditive variance in the breeding population of SCC is underway and it will allow sound decisions for the future.

CHAPTER 5 CONCLUSIONS

Within the last two decades, there has been increasing interest on the part of many forestry companies to plant *Eucalyptus* in order to satisfy the increasing demand for hardwood pulp in the international market. These companies have developed tree improvement programs for the species or its hybrids, and use clonal forestry as the main avenue for increasing productivity and product uniformity. For these programs the accurate estimation of basic genetic parameters is crucial for determining the best strategies for tree breeding and clonal forestry, as well as to predict genetic gains for breeding or from deploying the best material. Unfortunately, there are no estimates from multiple sites over many ages, and given the importance of *Eucalyptus grandis* in world plantations, these estimates are important.

The expansion of the forestry program with *E. grandis* at Smurfit Carton de Colombia (SCC) has led the company to the use of different and new environments where the performance of the species has not satisfied all the expectations. This study used data from 7 seedling trials and 7 clonal trials to develop accurate and precise information that allowed the company to make sound decisions in the tree improvement program to maximize genetic gains in a cost-effective manner.

This research has the advantage over previous studies of providing results at rotation age, six years, a characteristic of genetic trials not common in forestry due to the long rotation ages of most species. In addition, some of the multivariate analyses include models that combine clonal and seedling tests of inherent different genetic structures and with distinct statistical designs. These analyses were possible due to the flexibility of Mixed Model Methods that allowed simultaneous estimation of random and fixed effects from data of different sources, quality and quantity.

Based on the results of the previous chapters the main conclusions and implications of this study are the following:

1. Differences in survival, growth and the moderate type B genetic correlation for mean annual increment (MAI) between the different edaphoclimatic environments designed as “target environment”, “Guachicona” and “Maravillas” indicates that clonal testing should be conducted at each environment to maximize genetic gains.
2. The low to moderate genetic control exhibited by the clonal material for growth traits suggests that high genetic gains are obtainable for reasonable selection intensities over the target environment. However, the moderate type B genetic correlation in the target environment, indicates of the instability of clones across the range of sites commonly planted. The lack of correlation with the environmental variables associated with these sites means the clone by site interaction is not predictable. This precludes the use of particular clones in the specific sites where they perform best. Rather, it is important to

breed and test for widely adapted clones that perform stably across sites in the target environment.

3. The optimal selection age of three years for MAI based on age-age correlations and on the discounted selection efficiency at perpetuity facilitates increasing genetic gains per unit of time by reducing the generation interval for propagation and harvesting of the best clonal selections
4. Efficient clonal testing, using single tree plots, to maximize genetic gains from selection and deployment of the best clones was shown to be optimal by using between 36 and 40 ramets per clone, (through a combination of either 6 sites and 6 blocks or 10 sites and 4 blocks). This design allows the testing of 100 genetic entries at a spacing 3m x 3m in about one ha of land reducing the within block variance and the variance of the site which in turn increases the precision of parameter estimates for selection purposes.
5. The high type B genetic correlation observed for wood density in both the clonal and seedling material indicates little GxE for this trait. Further, low genetic correlations between growth traits and wood density as well as the high age-age correlation for wood density between three years and six years facilitates its use as a single trait or combined in a selection index with MAI.
6. The low genetic correlation between clones and seedlings from the same ortets indicates a lack of correspondence. This result, coupled with the moderate clone by site interaction in clones, means that all clones must be widely tested in the target environment before operational deployment. Further, breeding for

general combining ability in a recurrent selection program may not provide any gains to the clonal option if the main objective of the program is to deploy clones.

Future studies should be aimed to (1) identify the environmental factors that cause the genotype by environment interaction of clones in order to be able to increase genetic gains by planting clones where they perform the best and (2) screen the factors associated with the low correlation between clones and seedlings.

APPENDICES

APPENDIX 1
BROAD SENSE HERITABILITY (H^2) ESTIMATES FOR GROWTH TRAITS AND
WOOD DENSITY IN *EUCALYPTUS GRANDIS* (HILL) EX-MAIDEN

Trait	Age (years)	H^2 (S.E) ¹	Sites (No)	Number of Clones (n)	Author
Height	1.0	0.28	7	65	Endo and Wright 1993
Height	2.5	0.28	1	48	Kageyama
DBH ²		0.22			and Kikuti
Volume		0.26			1989
Height	1.0	0.30 (0.08)	2	50	Ikemori
		0.37 (0.09)		49	1990
DBH	1.0	0.24(0.07)	2	52	
		0.37(0.08)		53	
Height	3.0	0.37 (0.05)	4	460	Lambeth <i>et</i>
DBH		0.37 (0.04)			<i>al.</i> 1994
Volume		0.41 (0.04)			
Height	3.0	0.33	2	27	Lambeth <i>et</i>
DBH		0.34			<i>al.</i> 1994
Volume ³		0.41			
Wood density	7.5	0.30	1	27	Rockwood <i>et</i> <i>al.</i> 1995

¹ Standard error of the estimate

² Diameter at breast height

³ Individual tree volume

APPENDIX 2

PERCENT OF STEM BREAKAGE AND RESULTS FROM THE ANALYSES OF VARIANCE

Average stem breakage (% of broken-top trees) of three clonal tests from year two to six.

Location	Code	Damage (%)			
		3 year	4 year	5 year	6 year
Arcadia	1	9.6	11.5	13.9	16.0
Cedral	2	7.0	8.2	8.5	8.5
Maravillas	5	10.4	12.1	14.6	22.0
Average		9.0	10.6	12.3	15.6

Statistical Model for the Analysis of Variance Using Broken -top Trees as a Covariable

$$y_{ijklm} = \mu + B_j + c_k + bc_{jk} + d_l + bd_{jl} + cd_{kl} + e_{ijklm}$$

where

y_{ijklm} is the response variable, *i.e.* height, DBH/height

μ is the general mean

B_j is the fixed effect of the j^{th} block, with $j = 1, \dots, 8$

c_k is the random effect of the k^{th} clone, with $k=1, \dots, 67$ and $c_k \sim \text{NID}(0, \sigma_c^2)$;

bc_{jk} is the random interaction effect of the j^{th} block and k^{th} clone, with

$$bc_{jk} \sim \text{NID}(0, \sigma_{cb}^2);$$

d_l is the fixed effect of stem breakage;

bd_{jl} is the random interaction effect of the j^{th} block and l^{th} effect, with

$$bd_{jl} \sim \text{NID}(0, \sigma_{bd}^2);$$

cd_{kl} is the random interaction effect of the k^{th} clon and l^{th} effect, with

$$cd_{kl} \sim NID(0, \sigma^2_{cd});$$

e_{ijkl} is the random residual effect, with $w_{ijklm} \sim NID(0, \sigma^2_e)$.

Analyses of variance for total height (m) and the ratio DBH/height in the three clonal tests "Arcadia", "Cedral", and "Maravillas". Differences are established between non-damaged trees at three years and recovered trees in the following years.

Trait / Age / Source	Height				DBH/Height			
	3	4	5	6	3	4	5	6
	MSE ^a	MSE	MSE	MSE	MSE	MSE	MSE	MSE
<u>Arcadia</u>								
Bloque	116.12 **	7.69 ns	47.74 **	64.19 *	0.193 ns	0.126 *	0.074 ns	0.039 ns
Clon	14.62 ns	15.70 ns	38.64 ns	43.69 ns	0.107 ns	0.099 ns	0.224 ns	0.189 ns
Blo * Clo	4.63 **	6.77 **	11.47 **	13.11 **	0.044 **	0.038 **	0.085 **	0.058 **
BT	126.05 **	84.88 **	35.11 **	17.31 ns	0.487 *	0.001 ns	0.039 ns	0.019 ns
Blo*BT	10.62 **	7.52 **	1.00 ns	6.69 ns	0.062 **	0.019 ns	0.036 ns	0.008 ns
Clon*BT	9.15 **	6.52 **	8.57 **	10.84 *	0.044 **	0.021 ns	0.066 *	0.021 ns
<u>Cedral</u>								
Bloque	10.02 ns	20.00 *	26.45 ns	44.68 ns	0.049 ns	0.046 ns	0.053 ns	0.073 ns
Clon	9.06 ns	27.29 ns	49.52 ns	73.99 ns	0.105 ns	0.115 ns	0.109 ns	0.133 ns
Blo * Clo	4.56 **	8.06 **	18.67 **	26.33 **	0.042 **	0.067 *	0.054 **	0.073 **
BT	529.82 **	813.60 **	1573.20 **	1750.00 *	1.228 **	0.004 ns	0.073 ns	0.199 *
Blo*BT	5.29 **	5.05 ns	14.15 *	25.81 **	0.028 ns	0.027 ns	0.022 ns	0.025 ns
Clon*BT	4.22 **	9.83 **	17.33 **	24.80 **	0.037 **	0.044 **	0.037 **	0.052 **
<u>Maravillas</u>								
Bloque	1.58 ns	9.61 ns	14.28 ns	23.59 ns	0.037 ns	0.026 ns	0.027 ns	0.020 ns
Clon	4.75 ns	6.95 ns	14.00 ns	16.74 ns	0.065 ns	0.045 ns	0.060 *	0.057 ns
Blo * Clo	2.45 **	3.94 **	8.00 **	9.45 **	0.023 **	0.027 **	0.037 **	0.039 **
BT	180.09 **	137.62 **	244.68 **	324.17 **	0.989 **	0.016 ns	0.015 ns	0.018 ns
Blo*BT	4.61 **	3.20 ns	4.07 ns	7.13 ns	0.085 **	0.007 ns	0.011 ns	0.016 ns
Clon*BT	5.18 **	5.21 **	6.70 **	9.94 **	0.051 **	0.011 ns	0.009 ns	0.014 ns

^a Note: Mean square error (MSE) and significance of the F-test; * = significant at 5% probability value, ** = significant at 1% probability value, and ns = non-significant.

APPENDIX 3

SINGLE SITE AND ACROSS-SITE STATISTICAL MODELS FOR ESTIMATION OF GENETIC PARAMETERS FOR STEM BREAKAGE

Single-site Model

$$y_{jkl} = \mu + B_j + c_k + bc_{jk} + e_{jkl}$$

where

y_{jkl} is the observable trait

μ is the general mean

B_j is the fixed effect of the j^{th} block, with $j = 1, \dots, 8$

c_k is the random effect of the k^{th} clone, with $k=1, \dots, 65$ and $c_k \sim N(0, \sigma_c^2)$;

bc_{jk} is the random interaction effect of the k^{th} clone with the j^{th} block, with $cb_{jk} \sim$

$N(0, \sigma_{bc}^2)$; and

e_{jkl} is the random residual effect, with $e_{ijk} \sim N(0, \sigma_e^2)$.

Across-site Model

$$y_{ijkl} = \mu + S_i + B_{j(i)} + c_k + cs_{ik} + bc_{ijk} + e_{ijkl}$$

where

y_{ijkl} is the observable binomial trait

μ is the general mean

S_i is the fixed effect of the i^{th} site, with $i = 1, \dots, 3$

$B_{j(i)}$ is the fixed effect of the j^{th} block in the i^{th} site, with $j = 1, \dots, 8$

c_k is the random effect of the k^{th} clone, with $k=1, \dots, 67$ and $c_k \sim N(0, \sigma_c^2)$;

cs_{ik} is the random interaction effect between the i^{th} site and the k^{th} clone, with cs_{ik}

$$\sim N(0, \sigma^2_{cs});$$

bc_{ijk} is the random effect of the k^{th} clone with the j^{th} block, and i^{th} site, with $bc_{ijk} \sim$

$$N(0, \sigma^2_{cb}); \text{ and}$$

e_{ijkl} is the random effect of the l^{th} individual of the ijk^{th} plot

$$\text{with } e_{ijkl} \sim N(0, \sigma^2_e).$$

Formulae for Estimation of Genetic Parameters

$$\text{Single site} \quad H^2 = \frac{\sigma^2_c}{\sigma^2_c + \sigma^2_{bc} + \sigma^2_e}$$

$$\text{Pooled sites} \quad H^2 = \frac{\sigma^2_c}{\sigma^2_c + \sigma^2_{cs} + \sigma^2_{bkc} + \sigma^2_e}$$

Single-site and pooled site heritability for stem breakage, based on binomial data, of three clonal tests from year two to six.

Location	Code	Heritability			
		3 year	4 year	5 year	6 year
Arcadia	1	0.070	0.072	0.086	0.083
Cedral	2	0.077	0.087	0.091	0.091
Maravillas	5	0.072	0.093	0.076	0.101
Pooled		0.022	0.034	0.033	0.032

APPENDIX 4.
VARIANCE COMPONENTS, PAIRED-SITE BROAD SENSE HERITABILITIES
AND TYPE GENETIC CORRELATIONS OF GROWTH TRAITS

Variance components, paired-site broad sense heritabilities and type B genetic correlations of height (m) for the seven clonal tests at six years. The pair-site comparisons of "Guachicono" and "Maravillas" are separated from the others based on their lower heritabilities and type B genetic correlations.

Pairs of sites			σ_e^2	σ_s^2	σ_r^2	σ_e^2	σ_p^2	H_{BS}^2	r_B
Target environment									
Arcadia	1 - Cedral	2	0.1473	0.0977	0.1519	0.6377	1.0347	0.1424	0.6012
Arcadia	1 - Indostan	4	0.0782	0.0969	0.1037	0.7311	1.0099	0.0774	0.4465
Arcadia	1 - La Tulia	6	0.2392	0.1438	0.1353	0.5867	1.1050	0.2164	0.6245
Arcadia	1 - Suiza	7	0.2392	0.1438	0.1353	0.5867	1.1050	0.2164	0.6245
Cedral	2 - Indostan	4	0.1954	0.0595	0.1466	0.6465	1.0479	0.1864	0.7665
Cedral	2 - La Tulia	6	0.2117	0.1251	0.1684	0.5653	1.0704	0.1978	0.6286
Cedral	2 - Suiza	7	0.1816	0.1521	0.2139	0.4907	1.0382	0.1749	0.5442
Indostan	4 - La Tulia	6	0.3827	0.0577	0.1230	0.6050	1.1684	0.3275	0.8691
Indostan	4 - Suiza	7	0.2774	0.0477	0.2139	0.4776	1.0167	0.2729	0.8533
La Tulia	6 - Suiza	7	0.2254	0.1957	0.2376	0.3923	1.0510	0.2145	0.5353
Average								0.2027	0.6493
Guachicono									
Guachicono	3 - Arcadia	1	0.1792	0.1873	0.1255	0.5915	1.0835	0.1654	0.4890
Guachicono	3 - Cedral	2	0.0000	0.3299	0.1688	0.5657	1.0644	0.0000	0.0000
Guachicono	3 - Indostan	4	0.2762	0.1115	0.1099	0.6126	1.1101	0.2488	0.7125
Guachicono	3 - Maravillas	5	0.0000	0.2749	0.2133	0.5705	1.0587	0.0000	0.0000
Guachicono	3 - La Tulia	6	0.2569	0.2721	0.1578	0.3840	1.0707	0.2399	0.4856
Guachicono	3 - Suiza	7	0.2175	0.2074	0.2448	0.3808	1.0505	0.2071	0.5120
Average								0.1435	0.3665
Maravillas									
Maravillas	5 - Arcadia	1	0.0006	0.1313	0.1529	0.7223	1.0071	0.0006	0.0047
Maravillas	5 - Cedral	2	0.1793	0.0547	0.1760	0.6341	1.0441	0.1717	0.7662
Maravillas	5 - Guachicono	3	0.0000	0.2749	0.2133	0.5705	1.0587	0.0000	0.0000
Maravillas	5 - Indostan	4	0.0000	0.1280	0.1395	0.7420	1.0095	0.0000	0.0000
Maravillas	5 - La Tulia	6	0.0000	0.3051	0.1942	0.5695	1.0687	0.0000	0.0000
Maravillas	5 - Suiza	7	0.0120	0.2893	0.2512	0.4534	1.0059	0.0119	0.0398
Average								0.0307	0.1351

APPENDIX 5
AVERAGE SURVIVAL (%), AND BROAD SENSE HERITABILITIES BASED ON
0/1 DATA AND ON THE UNDERLYING SCALE FOR CLONAL TESTS IN THREE
DIFFERENT TYPES OF ENVIRONMENTS.

Age	Guachicono			Maravillas			Target environment		
	Survival (%)	H ² (0/1)	H ² Liability	Survival (%)	H ² (0/1)	H ² Liability	Survival (%)	H ² (0/1)	H ² Liability
1	84.35	0.014	0.032	94.77	0.011	0.047	95.07	0.011	0.049
2	81.53	0.190	0.403	82.30	0.079	0.170	93.13	0.035	0.128
3	80.00	0.202	0.412	76.97	0.136	0.261	92.61	0.035	0.124
4	77.81	0.202	0.393	76.04	0.135	0.255	91.93	0.044	0.145
5	72.56	0.280	0.500	72.80	0.133	0.239	91.35	0.038	0.120
6	65.90	0.286	0.478	71.65	0.132	0.234	89.85	0.036	0.104

APPENDIX 6. STATISTICAL MODELS FOR GENOTYPE-BY-ENVIRONMENT INTERACTION AND STABILITY INDEX

Linear Model

Statistical models for the linear regression of growth traits (height, individual tree volume and MAI), and the genotype-by-environment (GxE) interaction term on climatic factors, elevation (asl), mean annual temperature, and mean annual precipitation.

$$y_{ijkl} = \mu + e_i + p_j + t_k + w_{ijkl}$$

where

y_{ijkl} is the observable trait or GxE interaction term

μ is the general mean

e_i is the random effect of the i^{th} altitude, with $i = 1, \dots, 5$ and

$$e_i \sim N(0, \sigma_e^2);$$

p_j is the random effect of the j^{th} mean annual precipitation, with $j=1, 5$ and

$$p_j \sim N(0, \sigma_p^2);$$

t_k is the random effect of the k^{th} mean annual temperature, with $j=1, 5$ and

$$t_k \sim N(0, \sigma_t^2);$$

w_{ijkl} is the random residual effect with $w_{ijkl} \sim N(0, \sigma_w^2);$

Stability Index (Sierra, 1999)

$$\rho = \frac{\sum_{i=1}^5 (GxE)^2 clone_j}{(n/N) * \sum_{I=1}^5 \sum_{j=1}^{65} (GxE)^2}$$

where:

- ρ is the stability index corresponding to clone j^{th}
- GxE is the marginal predicted interaction term for clone j^{th}
- n is number of GxE interaction terms corresponding to clone j^{th} in sites one to five in the target environment.
- N is the total number of GxE interaction terms of all clones in all sites in the target environment.

APPENDIX 7
VARIANCE COMPONENTS FROM MULTIVARIATE ANALYSE S OF GROWTH
TRAITS

Variance components estimates of height from a multivariate analyses of "Guachicono", "Maravillas", and a target environment at different ages.

Locations	Age (years)					
	1	2	3	4	5	6
<u>Guachicono</u>						
σ_c^2	0.3647	0.4932	0.5033	0.6086	0.5537	0.5157
σ_b^2	0.1838	0.1465	0.1796	0.2198	0.1729	0.1377
σ_e^2	0.4671	0.4271	0.4094	0.2923	0.3437	0.3427
σ_p^2	1.0156	1.0668	1.0923	1.1207	1.0703	0.9961
<u>Maravillas</u>						
σ_c^2	0.1553	0.1592	0.0801	0.1031	0.0728	0.0897
σ_b^2	0.1621	0.1272	0.0939	0.1698	0.1830	0.2118
σ_e^2	0.6800	0.7246	0.8364	0.7588	0.7624	0.7274
σ_p^2	0.9974	1.011	1.0104	1.0317	1.0182	1.0289
<u>Target environment</u>						
σ_c^2	0.2343	0.1655	0.1088	0.1239	0.1456	0.2044
σ_s^2	0.0592	0.0918	0.0986	0.1454	0.1346	0.1228
σ_b^2	0.1881	0.2309	0.2194	0.1932	0.2198	0.1832
σ_e^2	0.5316	0.5148	0.5819	0.5681	0.5384	0.5416
σ_p^2	1.0132	1.003	1.0087	1.0306	1.0384	1.052

Variance components estimates of individual tree volume from a multivariate analyses of "Guachicono", "Maravillas", and a target environment at different ages.

Locations	Age (years)				
	2	3	4	5	6
<u>Guachicono</u>					
σ^2_c	0.3968	0.4072	0.4579	0.4365	0.4666
σ^2_t	0.1427	0.153	0.1414	0.1102	0.1197
σ^2_e	0.4771	0.4609	0.4201	0.4613	0.467
σ^2_p	1.0166	1.0211	1.0194	1.008	1.0533
<u>Maravillas</u>					
σ^2_c	0.1677	0.1632	0.1843	0.1815	0.1707
σ^2_t	0.1459	0.0835	0.0821	0.072	0.1044
σ^2_e	0.6971	0.7669	0.7571	0.777	0.7411
σ^2_p	1.0107	1.0136	1.0235	1.0305	1.0162
<u>Target environment</u>					
σ^2_c	0.1613	0.1503	0.1916	0.2228	0.2496
σ^2_s	0.0827	0.1007	0.1102	0.11	0.108
σ^2_r	0.1867	0.1428	0.1407	0.1416	0.1364
σ^2_e	0.582	0.6169	0.5803	0.5544	0.5411
σ^2_p	1.0127	1.0107	1.0228	1.0288	1.0351

Variance components estimates of mean annual increment (MAI) from a multivariate analyses of "Guachicona", "Maravillas", and a target environment at different ages.

Locations	Age (years)				
	2	3	4	5	6
<u>Guachicona</u> ¹					
σ_c^2	0.4581	0.4473	0.5088	0.4916	0.5096
σ_t^2	0.1726	0.2111	0.1564	0.1418	0.1085
σ_e^2	0.4041	0.374	0.3558	0.3787	0.3653
σ_p^2	1.0348	1.0324	1.021	1.0121	0.9834
<u>Maravillas</u>					
σ_c^2	0.1764	0.211	0.2224	0.192	0.2108
σ_t^2	0.1487	0.1497	0.1667	0.1739	0.1761
σ_e^2	0.6803	0.6606	0.6442	0.6573	0.6363
σ_p^2	1.0054	1.0213	1.0333	1.0232	1.0232
<u>Target</u> ²					
<u>environment</u>					
σ_c^2	0.1432	0.1384	0.1793	0.2057	0.225
σ_s^2	0.1032	0.1248	0.1296	0.1329	0.128
σ_r^2	0.2216	0.1965	0.1855	0.1726	0.1706
σ_e^2	0.5472	0.5608	0.5363	0.5208	0.5097
σ_p^2	1.0152	1.0205	1.0307	1.032	1.0333

Note¹: σ_c^2 , σ_t^2 , σ_e^2 , σ_p^2 are the variance among clones, variance of block by clone, variance of the residual and phenotypic variance, respectively.

Note²: σ_s^2 variance of site by clone and σ_r^2 variance of block within site by clone interaction.

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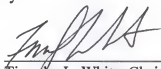
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BIOGRAPHICAL SKETCH

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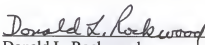
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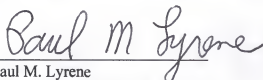
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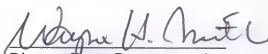
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